"SYNTHESIS, CHARACTERIZATION OF FLAVONOL DERIVATIVES AND IT'S BIODYNAMIC ACTIVITIES AND ASSOCIATED PARAMETERS"

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI.

In partial fulfillment of the requirements for the

Award of the degree of

MASTER OF PHARMACY
(PHARMACEUTICAL CHEMISTRY)

Submitted by

Reg. No: 26104232

Under the guidance of

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Principal

MAY- 2012

NANDHA COLLEGE OF PHARMACY & RESEARCH INSTITUTE

ERODE – 638052, TAMIL NADU

CERTIFICATE

DECLARATION

ACKNOWLEGDEMENT

CONTENT

CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"Synthesis, Characterization Of Flavonol Derivatives And It's Biodynamic Activities And Associated Parameters"** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for the partial fulfillment of the degree of "Master of Pharmacy" in Pharmaceutical Chemistry was carried out by **(Reg. No. 26104232)** in the Department of Pharmaceutical Chemistry, Nandha College of Pharmacy and Research Institute, Erode-52, in under my direct supervision and guidance.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any university and the dissertation represent entirely an independent work on the part of the candidate.

Date**:**

Place: Erode

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DECLARATION

The work presented in this thesis entitled **"Synthesis, Characterization Of Flavonol Derivatives And It's Biodynamic Activities And Associated Parameters"** was carried out by me in the Department of Pharmacology, Nandha College of Pharmacy and Research Institute, Erode-52 under the direct supervision of **Dr. T. Sivakumar, M. Pharm., Ph.D.,** Principal, Nandha College of Pharmacy and Research Institute, Erode-52.

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any University.

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Vivek and **Shahu** who always stood with me in my success and failure and motivated me to the path of victory.

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ABBREVIATIONS

CMC - Carboxy Methyl Cellulose

DMF - Dimethyl Formamide

DMSO - Dimethyl Sulfoxide

Glut - Glucose Transporter

h - Hours

hb - Hemoglobin

IR - Infra Red

Mg - Milligram

Ml - Milliliter

Mm - Millimeter

MS - Mass Spectroscopy

NMR - Nuclear Magnetic Resonance

p.o - Per oral

SAR - Structural Activity Relationship

TLC - Thin layer chromatography

UV - ultra visible

µg - microgram

1. INTRODUCTION

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Medicinal chemistry and pharmaceutical chemistry are disciplines at the intersection of chemistry, especially synthetic organic chemistry, and pharmacology and various other biological specialities, where it is involved with design, chemical synthesis and development for market of pharmaceutical agents (drugs)[1]. Just as in all fields of science, the history of medicinal chemistry is comprised of the ideas, knowledge and available tools that have advanced contempory knowlegde[2]. The pharmaceutical chemistry is being concerned primarily with modification of stuctures having known physiological or pharmacological effects with analysis of drugs.

No discussion of the evolution of medicinal chemistry would be complete without briefly mentioning combinatorial chemistry and high though out screening. Significant advances in X-rays crystallography and nuclear magnetic resonance have made it possible to obtain detailed representatives of enzymes and other drug receptors. Statistical methods based on the correlation of physiological properties with biological potency are used to explain and optimize biological activity.

To provide an understanding of principles of medicinal chemistry, it is necessary to consider the physiological properties used to develop new pharmaceutically active compounds and their mechanism of action, the drugs metabolism including possible biological activities of metabolites, the importance in stereochemistry in drug design and methods used to determine what “space” a drug occupies[1].

The earliest drug discoveries were made by random sampling of higher plants, producing hundreds and thousands of new organic chemicals are prepared annually throughout the world, and many of them are entered into pharmacological screens to determine whether they have useful biological activity. Most recently, auromated high throughput screening systems utilizing cell culture systems with linked enzyme assays and receptor molecules derived from gene cloning have greatly increased the efficiency of random screening. It is now practical to srceen enormous libraries in a short period of time, obtained from combinatorial chemistry procedures, by the development and use of robotics and automation[1].

Flavonoids class of natural products has found significant role in pharmaceutical effects including leishmanicidal activity, anti HIV, vasodilator, antiviral, antioxidant, anti inflammatory[3]. Still chemical modification of drug molecules to locate the member of the series having optimal effect has long been carried out, widely and will probably continue to be factor necessary to drug discovery[4].

Flavonoids are groups of polyphenolic compounds which are widely distributed throughout the plant kingdom. To date about 3000 varieties of flavonoids are known. Many have low toxicity in medicine for maintainance of capillary integrity[8]. They are integral components of comman diet present in fruits, vegatables, olive oil, tea and red wine. It was demonstrated that flavonoids posses anxiolytic, anti-inflammatory, antiviral, antiprotozoal and anticarcinogenic activities. Moreover, a number of studies have suggested that flavonoids may display a protective role in the prevention of cancer, coronary heart diseas, bone loss and many other age related disease.

This of plants pigment in most angiosperm families, largely responsible for the colours including all parts of the plant and have important role in the growth and development of plants, protection against UV-B radiation forming antifungal barriers, antimicrobial, insecticidal estrogenic activities and helps in plant reproduction[5]. They are produced as a result of stressors that include climate, UV radiation, herbivorous and pathogen[6]. Over 4000 flavonoids compounds have been characterized and classified according to chemical structure[5]. Therefore, a general synthetic method and combinatorial synthesis of these molecules would be desirable. In recent years, scientific and public interest in flavonol has grown enormously due to their putative beneficial effects against atherosclerosis, osteoporosis, diabetes mellitus and certain cancer. Flavonols intake in the form of dietary supplements and plant extracts has been steadily increasing[7].

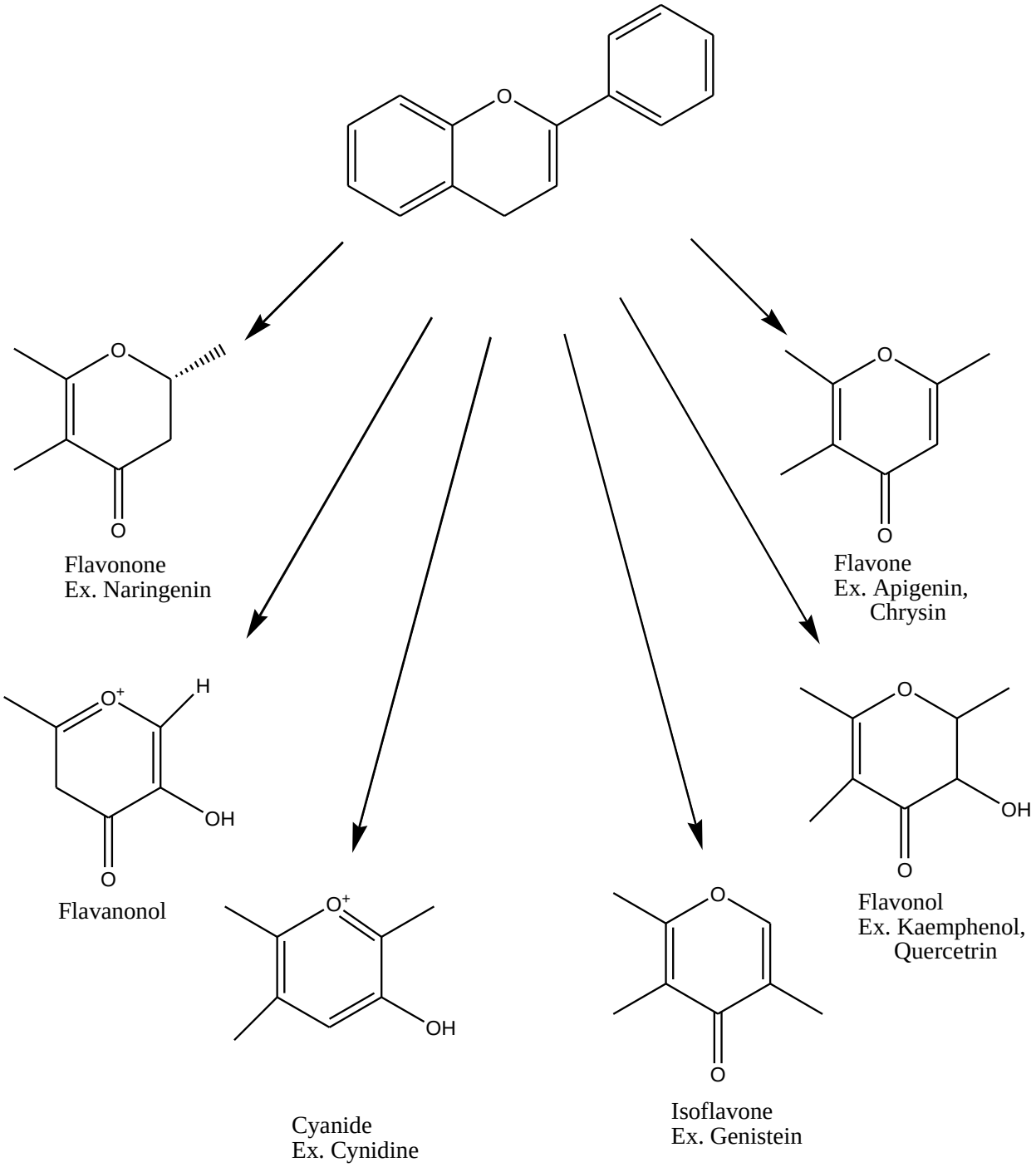
1.1 BASIC NUCLEUS:

Heterocyclic compounds are containing a ring structure with atoms in addition to carbon, such as sulphur, oxygen or nitrogen, as a part of ring. They may either have simple aromatic or non-aromatic ring, with a wide range of biological properties. The unique position occupied by heterocyclics in life processes has attracted much attention in the chemical, biochemical, medical, agriculture science and cognate branch of science and technology.

Flavonoids occurs as aglyclone, glycosides and methylated derivatives. In plants, flavonoids aglycones (i.e- flavonoids without attached sugar) occur in a variety of structural forms. All contain fifteen carbon atoms in their basic nucleus: two six membered rings linked with a three carbon unit which may or may not be a part of a third ring[10]. The flavonoid aglycone consists of a benzene ring condensed with a six membered ring, which in the 2-position carries a phenyl ring as a substituent. The six member ring condensed with the benzene ring is either a ϒ-pyrone (flavonols and flavonones) or its dihydroderivative (flavanols and flavanones). The position of the benzenoid substituent divides the flavonoid class into flavonoids (2-position) and isoflavonoids (3-position). Flavonols differ from flavonones by hydroxyl group the 3-position and a C_2-C_3 double bonds[9]. Flavonoids are often hydroxylated in position 3, 5, 7, 2', 3', 4', 5'. Methylethers and acetylesters of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, glucor-hamnose, galactose or arabinose[11].

Chromones react with electrophiles at the exocylics oxygen atom, and can be cleaved by reaction with sodium hydroxide, ammonia and other nucleophiles (eg- reaction with hydrazine). Flavonoids dissolve in basic solution, resulting in a yellow color, which increases with pH anh the number of hydroxyl griups, but appears to be colorless solution upon addition of acid. It is soluble in sulphuric acid and it forms crystalline hydrochloride, when hydrogen chloride is passed into an ethereal solution of compounds[11].

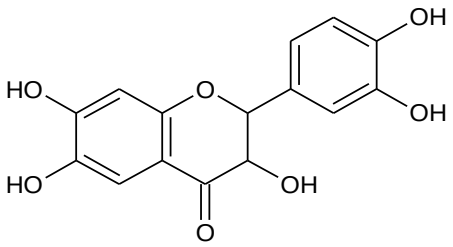
Flavonoids may be divided into six different major classes (flavones, isoflavones, flavonols, flavononols, flavonones and anthocyanidins), this is based on differences in molecular backbone structure[8].


O
O
O
Flavonone
Ex. Naringenin
O
O
Flavone
Ex. Apigenin,
Chrysin
O+
H
OH
O
Flavanonol
O+
OH
Cyanide
Ex. Cynidine
O
O
Isoflavone
Ex. Genistein
O
OH
O
Flavonol
Ex. Kaemphenol,
Quercetrin

1.2 STRUCTURE ACTIVITY RELATIONSHIP:

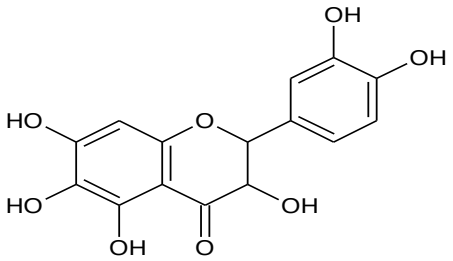
Flavanoids are benzo-γ-pyrone derivatives consisting a benzene ring-A and B comprising oxygen containing pyran ring C. The authors propose that small structural differences in the compounds are critical to their activity and their low toxic potential. Several structural determinants for flavanoids have been proposed include:-

- The *O*-dihydroxy (catechol) structure in ring-B, which is the target of radicals for all the flavanoids with a saturated 2, 3-double bond.



- The 2, 3-double bond in conjugation with a 4-oxo function which is responsible for electron delocalization from the ring -B.

- The additional presence of both 3 and 5 hydroxyl groups for maximum radical scavenging potential and strong radical absorption[12].



- The presence of 5, 7, 4' hydroxyl group along with double bond between C_2 and C_3 and presence of oxo group at C_4 is essential for Vascular relaxation[13].
- The order of potency for Vascular relaxation was Flavones > Flavonols > Isoflavones > Flavonones > Flavononol > Chalcones[13].
- The hydroxyl group on 3' and 4' position of ring A possessed high hydroxyl scavenging activity but ring B having more than ring A[14].
- The hydroxyl group or glucoside on 3 position of ring C also has scavenging ability[15].
- In Flavonols, Galagin introduction of 4' – Fluorine, 2' – Chlorine, 4' – Chlorine not improves but introduction of 4' - Iodine increases the Anti Cancer activity[15].

- The distance between A and C rings should be 6.9 A^o including 4 – oxo and 7 – hydroxyl moieties for Anti-inflammatory activity[16].
- Replacing the –OH groups with –OCH3 or -COOCH3 groups will cause complete loss of activity[17].
- Flavanoids lacking –OH groups on their B ring are more active against microorganisms than those with the –OH groups; this findings supports the idea that their microbial target is the membrane.
- The presence of methoxy group at 4' position shows Anti-hypergylcemic activity[18].
- The presence of hydroxyl groups at 2', 3', 4', 3, 6, 7, and 8 shows good Anti-hypergylcemic activity.
- To enhance free radical scavenging activities of Flavonoids two structural factors are essential[18],
  a) Catechol moiety is necessary.
  b) Presence of double bond between $C_2 – C_3$ and C_4 – oxo function in ring C, namely pyrone moiety.

Literature survey reveals that flavan-4-ones are well known for their varied pharmacological and microbiological activities. The high structural specificities makes flavanols an interesting class of molecules for medicinal chemistry study, since the structural units have a profound effect on biological activity[15]. In view of the above mentioned facts and continuation of our work on the synthesis of biologically important heterocyclic compounds, research is divided into two parts.

Part I. Focuses on the synthesis of various 3-hydroxy flavone derivatives starting from commercially available starting materials. All the compounds synthesized were characterized by various spectroscopic methods i.e.: infrared (IR), ultraviolet (UV), mass spectroscopy (MS) and nuclear magnetic resonance (1H and 13C)

Part II. On the anti bacterial, antiinflammatory and antidiabetic properties and the effect of synthesized 3-hydroxy flavone which will be studied.

1.3 DIABETES MELLITUS:

Diabetes Mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration – hyperglycaemia (fasting plasma glucose > 7.0 mmol/l, or plasma glucose > 11.1 mmol/l 2 hours after a meal) – caused by insulin deficiency, often cobined with insulin resistance[19]. The risk of diabetic complication includes many particularly cardiovascular diseases, peripheral vascular disease. Complications such as coronary artery disease, strke, neuropathy, renal failure, retinopathy amputations and blindness are known to be associated with Diabetes Mellitus. Hyperglycaemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscle with reduced glycogen synthesis[20].

Diabetes Mellitus is a chronic condition characterized by major derangements in metabolism of glucose and abnormalities in metabolism of fat, protein. Presently there are different groups of oral hypoglycaemic agents for clinical use, having characteristic profiles of side effects. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with antidiabetic activity having fewer side effects.

1.3.1 CLASSIFICATION OF DIABETES MELLITUS:

Insulin dependent Diabetes Mellitus (IDDM-Type I)

Type 1 diabetes is a autoimmune disease. In diabetes, the immune system attacks the insulin producing beta cells in the pancreas and destroys them. It is clearly associated with all absolute deficiency of insulin secretion. The patients are usually young (children or adolescents) and not obese when they first develop symptoms. Viral infection may damage pancreatic beta cells and expose antigens that initiate a self perpetuating autoallergic process[21.]

Non-Insulin dependent Diabetes Mellitus (NIDDM-Type II)

The most comman form of diabetes is type 2 diabetes. About 90 to 95 % of people with diabetes have type 2. Non insulin dependent Diabetes Mellitus usually occurs after the age of forty[22]. This form of diabetes is associated with older age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity and ethnicity. About 80 % of people with type 2 diabetes are overweight. Type 2 diabetes is accompanied both by

insulin resistance and by impaired insulin secretion, each of which are important in its pathogenisis[19].

1.3.2 RISK FACTOR FOR TYPE 2 DIABETES [23]:

Obesity

Greater weight means a higher risk of insulin resistance, because fat interferes with the body's ability to use insulin.

Sedentary Lifestyle

The Surgeon General's Report on Physical Activity and health (USA, 1996) states that "a sedentary life style is damaging to health and bears responsibility for the growing obesity problems".

Unhealthy Eating Habits

Unhealthy eating contributes largely to obesity. Too much fat, not enough fiber and too many simple carbohydrates all contribute to a diagnosis of diabetes.

Family History and Genetics

It appears that people who have family members who have been diagnosed with type 2 diabetes are at a greater risk for developing it themselves.

Increased Age

Scientists theorize that the pancreas ages right along with us, and doesn't Pump insulin as efficiently as it did when we were younger. Also, as our cells age, they become more resistant to insulin as well.

High Blood Pressure and High Cholesterol

These two bad boys are the hallmark risk factors for many diseases and conditions, including Type 2 diabetes. Not only do they damage your heart vessels but they are two key components in metabolic syndrome, a cluster of symptoms including obesity, a high fat diet and lack of exercise. Having metabolic syndrome increases your risk of heart disease, stroke and diabetes.

History of Gestational Diabetes

Gestational diabetes affects about 4 % of all pregnant women. It begins when hormones from the placenta make the mother insulin resistant. Many women who have gestational diabetes develop type 2 diabetes years later. Their babies are also at some risk for developing diabetes later in life.

1.3.3 REGULATION OF GLUCOSE METABOLISM:

The facilitated diffusion of glucose into cells along a downhill gradient is ensured by glucose phosphorylation. This enzymatic reaction, the conversion of glucose to glucose-6-phosphate, is accomplishedby one of a family of hexokinases. The four hexokinases are distributed differently in tissues, and two are regulated by insulin. Hexokinase IV, a 50,000 dalton enzyme more commonly known as glucokinase gene, is found in association with GLUT 2 in liver and pancreatic beta cells. There is one glucokinase gene, but different first exons and promoters are employed in the two tissues. The liver glucokinase gene and Hexokinase II is regulated transcriptionally by insulin[24].

1.4 INFLAMMATION:

1.4.1 Definition

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is body defense reaction in order to remove the consequent necrosed cells and tissues well as to eliminate or limit the spread of injurious agent. Inflammation is a usual tissues reaction to physical, chemical or biological stress or injury. It manifests as an immunological response and amassing of inflammatory cells and play a role in both normal repair reactions and in the pathogenesis of disease. The inflammatory reactions are usually defined as acute or chronic on the basis of both their temporal duration and the prevailing phenomena. Inflammation is the body's effort to inactivate or destroy invading organism, remove irritants and set the stage for tissue repair[25].

The agent causing inflammation may be as order-

1) Physical Agents like heat, mechanism trauma, cold, radiation.
2) Chemical Agents like organic and inorganic poisons.
3) Infective Agents like bacteria, viruses and their toxins.
4) Immunological Agents like antigen antibody and cell mediated reaction.

1.4.2 Signs of Inflammation[26]

Five important signs of inflammation are,

1) Rubor (redness) 2) Calor (heat) 3) Tumor (swelling)
4) Dolor (pain) 5) Laesa (loss of function)

1.4.3 Causes

- Burns
- Chemical irritants
- Frostbite
- Toxins
- Infection by pathogen
- Necrosis
- Physical injury, blunt or penetrating
- Immune reactions due to hypersensitivity

- Ionizing radiation
- Foreign bodies, including splinters and dirt.

1.4.4 Types of Inflammation[27]

Depending upon the defence capacity of the host and duration of response, inflammation can classified as Acute and Chronic.

A) Acute Inflammation:

Infected ingrown toenail showing the characteristic redness and swelling associated with acute inflammation. Acute inflammation is a short-term process which is characterized by the classic signs of inflammation- swelling, redness, pain, heat and loss of function- due to the infiltration of the tissues by plasma and leukocytes. It occurs as long as the injurious stimulus is present and ceases once the stimulus has been removed, broken down, or walled off by scarring (fibrosis). The process of acute inflammation is initiated by the blood vessels local to the injured tissue, which alter to allow the exudation of plasma proteins and leukocytes into the surrounding tissue. The increased flow of fluid into the tissue causes the characteristic swelling associated with inflammation, and the increased blood flow to the area causes the reddened color and increased heat.

B) Chronic Inflammation:

Chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissues destruction, and attempts at repair. Chronic inflammation is not characterized by the classic signs of acute inflammation listed above. Instead, chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes and plasma cells), tissue destruction, and attempts at healing, which include angiogenesis and fibrosis. Endogenous causes include persistent acute inflammation. Exogenous causes are varied and include bacterial infection, especially by *Mycobacterium tuberculosis*, prolonged exposure to chemical agents such as silica, or autoimmune reactions such as rheumatoid arthritis.

1.5 BACTERIAL INFECTIONS:

In developing countries low levels of hygiene and sanitation exposed the susceptibility of people to opportunistic bacterial and fungal infections that increased dramatically the incidence and complications. Since last decade, there has been an increasing evidence of bacterial and fungal infections which proves the most critical problem due to population explosion, pollution, changed environmental conditions, wastes from different sources, which may affect food with perfect nutrition value. These factors cause less immunogenicity in human beings and animal[28]. Although prophylaxis and treatment strategies have been improved over the years, infections still contribute significantly to morbidity and mortility[29]. Owning to many advances in surgery, cancer treatment, solid organ and bone marrow transplantation, HIV epidemic and immune compromised patients, the severity has been still increasing[30].

Also multiple drug resistance developed in microorganism has significantly been increased in recent years. Less immunogenicity coupled with the resistance of microbes to antibiotics with increased toxicity in humans being and animal during prolonged the treatment with several antimicrobial drugs. Therefore, the flavonoids may be promising new class compounds in antimicrobial therapy. The use of flavonoids against bacterial and fungus infections has two purposes:

1) To kill the bacteria or fungus cells and
2) To counteract the spread and the effects of the bacterial toxins.

Microbes producing extended spectrum β- lactamases, that are resistant to virtually all β- lactam have been reported (Methicillin – Resistant Staphylococcus auresus, Vancomycin – Resistant Enterococci, Antibiotic – Resistant Gram – Negative Bacilli), for these kind of microbes, flavonoids may be considered potential for these infections in the future.

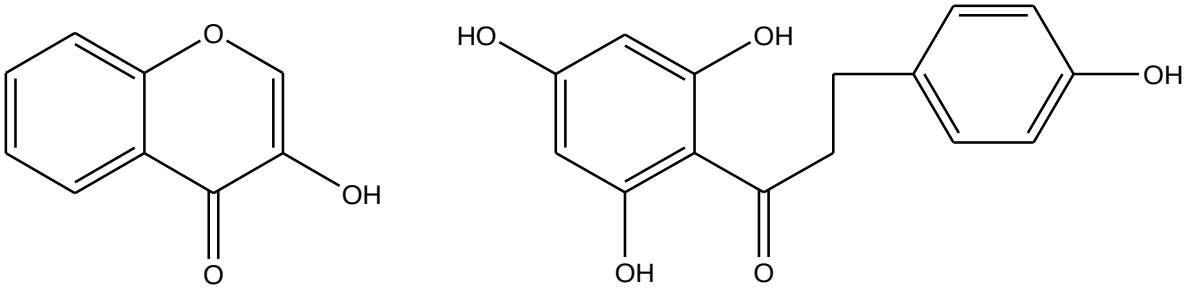
The most comman microorganism responsible for disease include:-

Gram – positive bacterial species	*Gram – negative bacterial species*
<u>Staphylococcus Spp:</u> *S.aureus, S.epidermidi, S.albus* *<u>Bacillus Spp:</u>* *B.cereus, B. fragile, B.subtilis,* *B.licheniformis* *<u>Strepyococcus Spp:</u>* *S.pyrogen, S.viridans, S.pneumoniae,* *S.baris, S.jaccali*	*Pseudomonas aeruginosa,* *Shigella boydii,* *K.pneumonia,* *B.cepacia,* *Salmonella typhi* *Escherichia coli.*

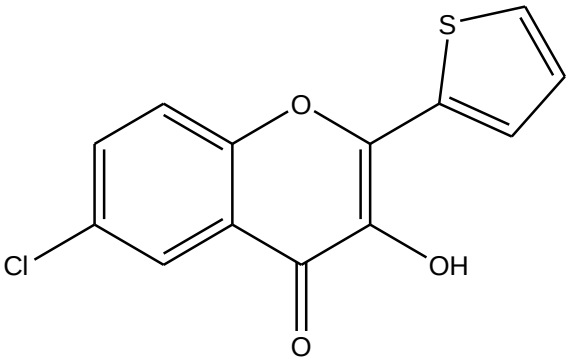
Many but not all of the bacterial strains commonly encountered by humans are killed by flavonoids. Activities of flavonoids are more potent against Gram-positive bacterial species than Gram-negative bacteria[31].

2. LITERATURE REVIEW

Narayana *et al*., synthesis flavonoids as antioxidant and anti hepatotoxic agents. The synthesized compounds were screened for their free radical scavenging abilities[32].

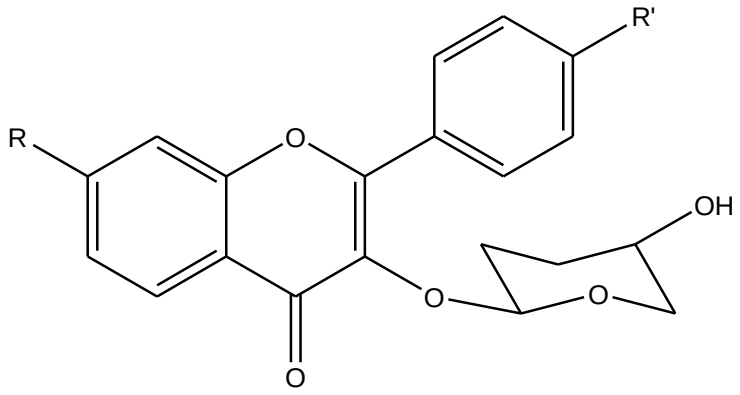


Bansal *et al*., synthesis and anti-diabetic as well as antispasmodic activity of 6-chloro-3-hydroxy-2-(2'-thienyl)-4-oxo-4H-1-benzopyran. The synthesized compounds were screened for their anti-diabetic and antispasmodic activity[33].



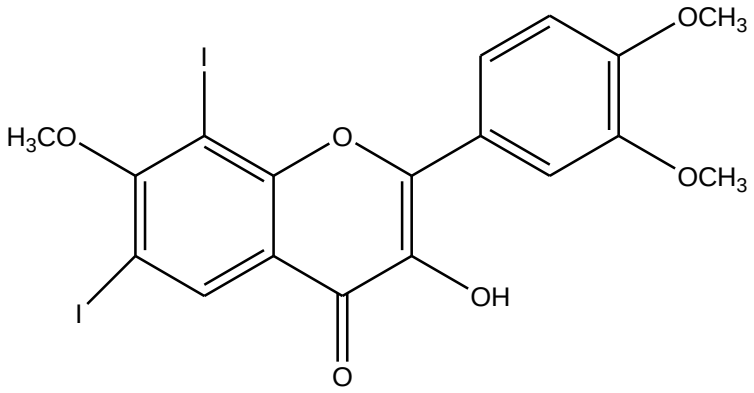
Li *et al*., synthesized libraries of flavonols made through Algar-Flynn-Oyamada reaction from 2'-hydroxyacetophenone and benzaldehyde also studied for their potential pharmacological and biological activities of these compounds[34].

R – R' = H, CH_3, OCH_3



Olaleye *et al.*, synthesis and anti-inflammatory as well as anti-nociceptive properties of flavonoids. The synthesized compounds were screened for their anti-inflammatory and anti-nociceptive activity[35].

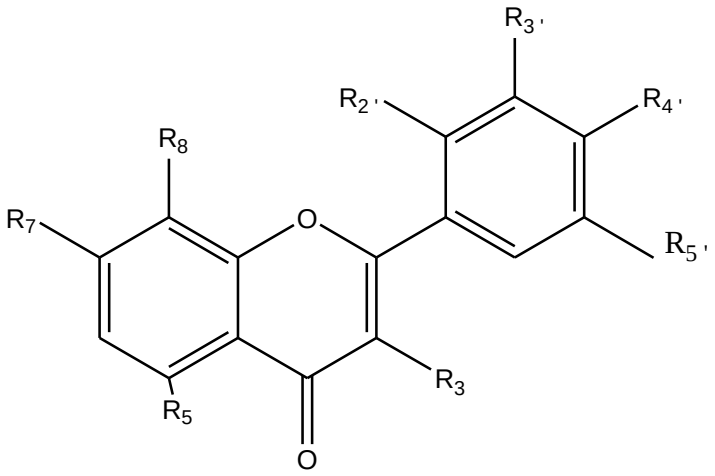
Carvalho *et al.*, synthesis of new iodine derivatives of flavonol and isoflavone by using iodine, potassium hydroxide and methanol[36].



Tapas *et al.*, synthesis flavonoids as nutraceuticals. The synthesized compounds were screened for their anti-microbial and anti-bacterial activity[37].

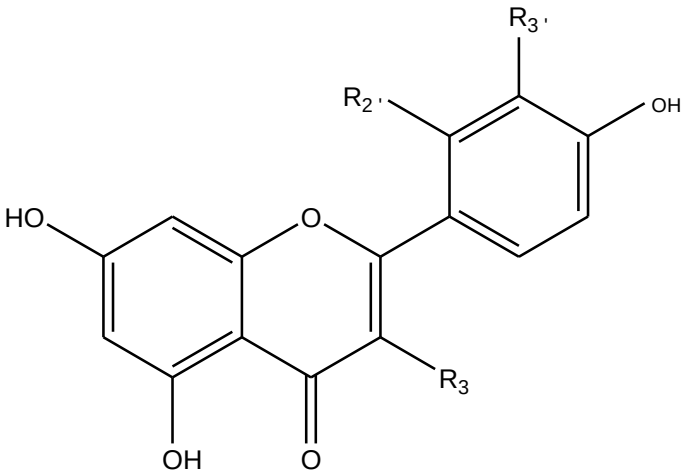
Amic *et al.*, described the relationship between the structural characteristics of flavonoids and their anti-oxidant activity and suggested the free radical scavenger potential of these polyphenolic compounds, also possible mechanism of action of flavonoids lacking B rings OHs as free radical scavengers has been proposed[38].

$R_3 – R_8$ = H, OH, Ogl $R_{2'} – R_{5'}$ = H, OH, OCH_3, Ogl

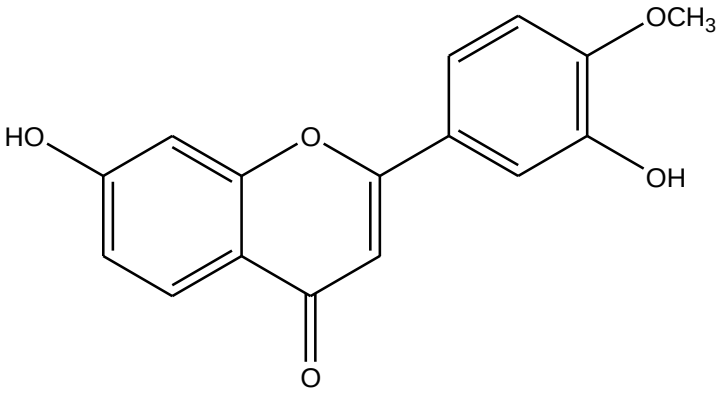


Alvarenz *et al*., has studied synergism of some substituted flavonoids along with anti-microbial activity and established relationships between membranes structures of various microorganisms and effects of the compounds[39].

R_3 = OH, O-Rut $R_{2'} – R_{3'}$ = OH

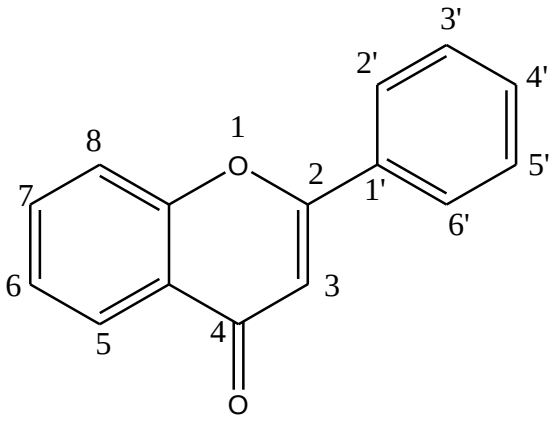


Xue *et al.*, reported the first total synthesis of (±)-3',7'-dihydroxy-4'-methoxyflavan, a naturally occurring flavan[40].



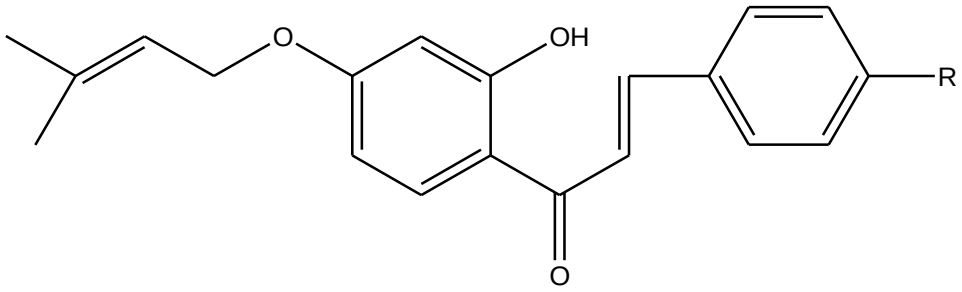
Cushnie *et al.*, studied anti-microbial activity, anti-viral activity and anti-fungal activity of flavonoids, including mechanism of action of various flavonoids[30].

2 – 6 = OH, OCH_3, Cl 2' – 6' = OH, OCH_3, Cl



Cespedes *et al.*, synthesis and anti-inflammatory properties of flavonol from Aristotelia chilensis extracts. The synthesized compounds were screened for anti-inflammatory activity[41].

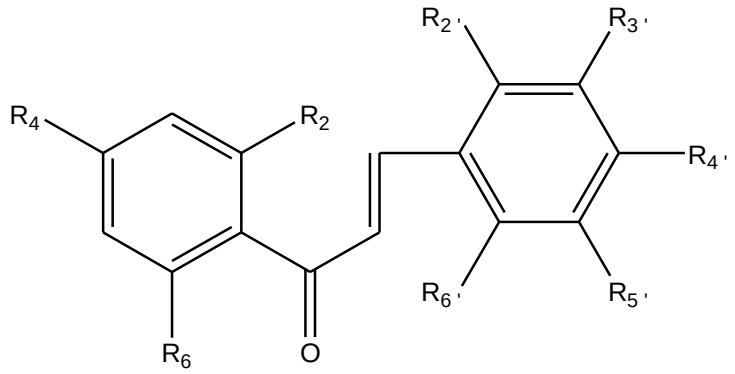
Bhsakar *et al.*, synthesis, characterization and anti-bacterial activity of new series of prenyloxy chalcones and prenyloxy flavonones. The synthesized and tested for anti-bacterial effects against E.coli, S.auresus[42].



Bylka *et al*., has presented a review of flavonoids that have a proven inhibitory activity against a variety of human pathogens, including anti-biotic resistant Gram-positive and Gram-negative strain of bacteria and viruses[43.]

Boumendjel *et al.* studied the anti-mitotic and anti-proliferative activity of chalcones[44].

$R_2 – R_6$ = H, OH, OCH_3, OC_2H_5, F, Cl, NH_2 $R_{2'} – R_{6'}$ = H, OH, OCH_3, OC_2H_5, Cl, NH_2



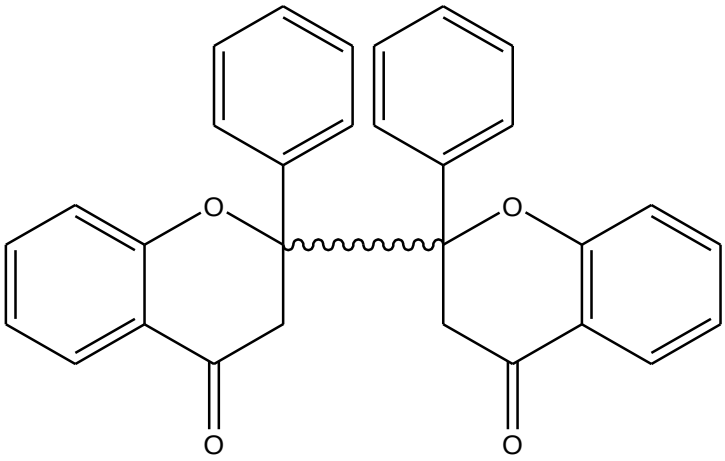
Nambi *et al.,* have reported the anti-inflammatory activity of flavones and its hydroxy derivatives[45].

Conti *et al.,* has reported the mechanism of action of the anti-rhinovirus flavonoid 4',6-Dicyanoflavan by inhibiting an early event of rhinovirus type 1B replication in Hela cells and suggested the stabilizing effects of Dicyanoflavan on virion capsid conformation is responsible for uncoating inhibition[46].

Jayasree *et al.,* has reported the anti-oxidant and anti-bacterial activity of new 3- methyl flavones[47].

Sivakumar *et al.,* has studied the anti-tumor activity and anti-oxidant status of Caesalpinia bonducella against Ehrlich ascites carcinoma in swiss albino mice[48].

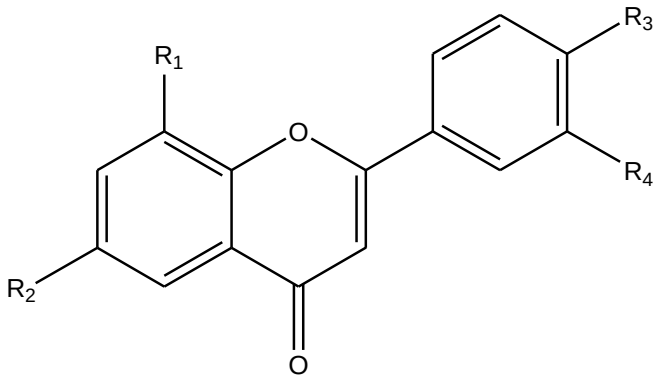
Chen *et al.,* has reported photo chemical synthesis of 2,2'-Biflavonones from flavones with their anti- HIV, anti-viral activities[49].



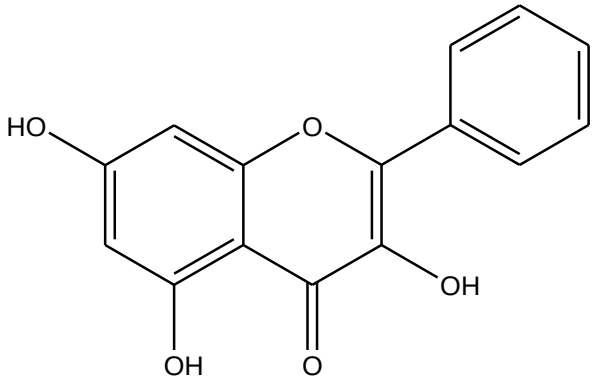
Mewett *et al.,* has reported synthesis and positive modulatory activities of a small library of flavan-3-ol derivatives on $\alpha_1 \beta_2 \Upsilon_{2I}$ $GABA_A$ receptor[50].

Ghotekar *et al.,* described synthesis of biologically important chromones and pyrazolines[51].

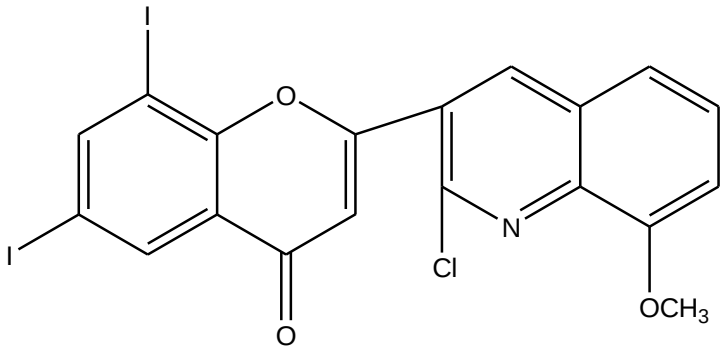
$R_1 - R_4$ = H, CH_3, OCH_3, Cl, F



Gorduza *et al*., syudied the structural reactivity relationship of anti-oxidant flavonoids suggesting anti-oxidant properties of hydroxyl flavones can be used in therapy of diseases generated or increased by radical mechanism oxidation and accumulation of radical O_2[52].

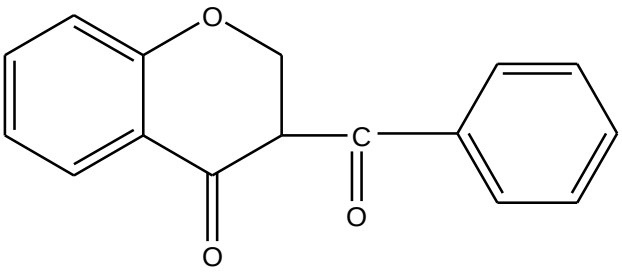


Vibhute *et al.,* has reported new chalcones derivatives synthesis from 2-chloro-8-methoxy-quinoline-3-carbaldehyde and halo hydroxyl substituted acetophenones via Clasien-schmidt screened for their anti-bacterial activity[53].

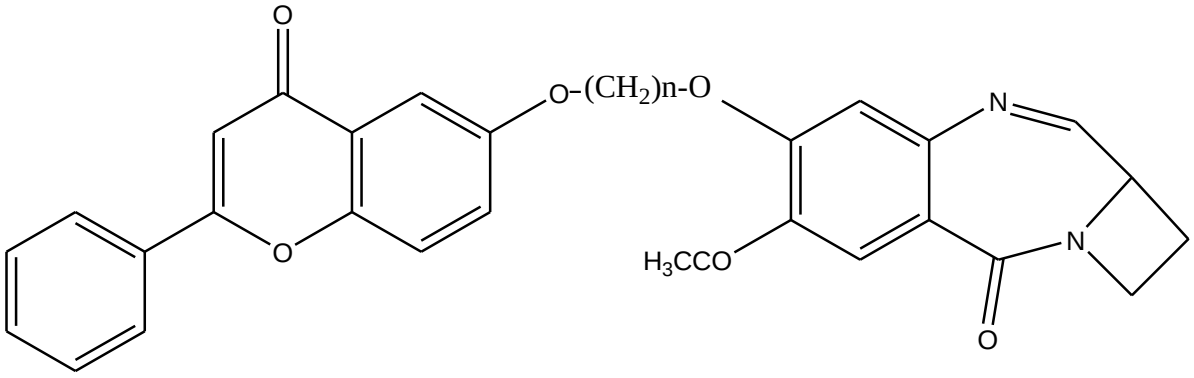


Sharma *et al*., has reported synthesis of dihydroxy chalcone derivative by microwaves. The synthesized compounds were screened for their anti-bacterial, anti-malarial and anti-mycobacterial activities[54].

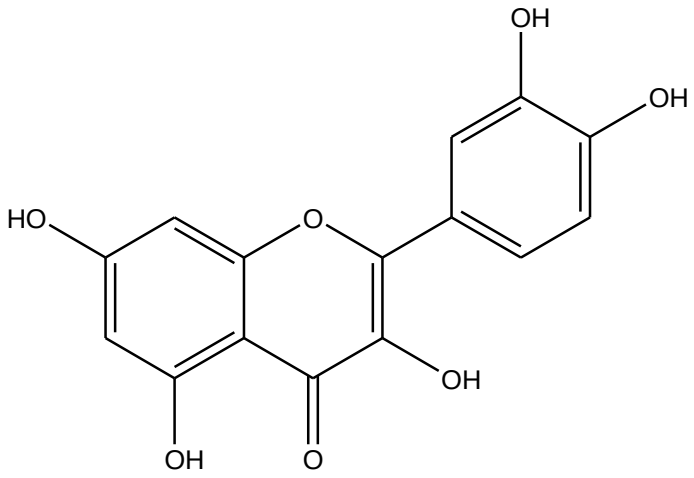
Wankhade *et al*., has reported synthesis and anti-oxidant activity of some 3-Aroyl chromanone and Flavanones[55].



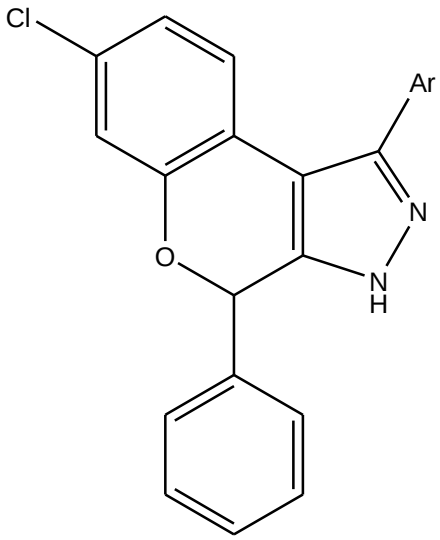
Kamal *et al*., has studied the synthesis and evaluation of new pyrrole[2,1-c] [1,4] benzodiazepine hybrids linked to a flavones moiety exhibiting significant DNA minor groove binding ability[56].



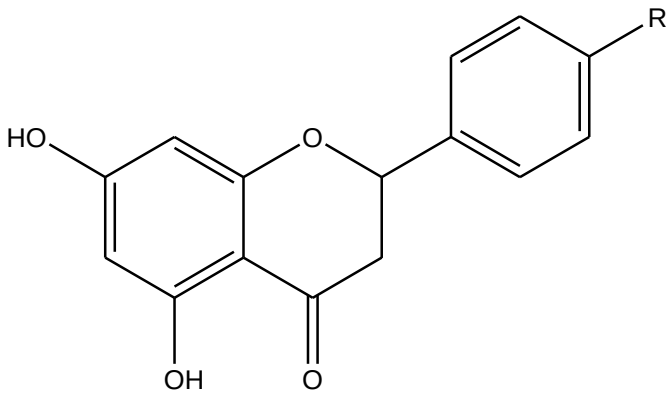
Witezak *et al.*, studied the synthesis of analogues of various of natural C-glycosyl compounds : C-glycosyl flavonoids, Multustriatin, Hernandulcin and (1-4)-C- linked disaccharides from the new carbohydrate synthone levoglucosenone[57].



Pattan *et al*., has reported synthesis and biological evaluation of some new benzopyrones[58].



Zhao *et al.*, reported synthesis and anti-depressant activites of series of 5,7-dihydroxy flavanones derivatives[59].



Venkatesan *et al*., reported synthesis and anti-microbial activity of substituted chromone derivatives[60].

Tachakittirugrod *et al.,* reported anti-oxidant activity of flavonoids isolated from Psidium guajava[61].

Marcel *et al.,* reported anti-oxidant activity of flavonoids extracted from leaves and stems of Adenia lobata[62].

3. AIM AND OBJECTIVE OF THE WORK

Current approaches in the treatment of hyperglycemia involve drug treatment with various classes of anti-diabetics. Major drawbacks of chemotherapy include adverse effects and drug resistance. Adverse effects associated with traditional anti diabetic drugs are hepatotoxicity and severe hypoglycemia. Therefore to overcome the shortcomings of the present treatment, an anti hyperglycemic drug with a new mechanism of action, capable of providing complete and safe treatment of diabetics mellitus.

Drug designing is an important tool in the field of medicinal chemistry wherein the syntheses of new medicinal compounds are done by molecular or chemical manipulation of the lead moiety in order of producing a highly active compound. Thus, making gradual changes in physio-chemical properties of the lead moiety to enhance the biological activities.

Literature survey reveals that 3-Flavonols are well known for their varied pharmacological activities. In view of the above mentioned facts and continuation of our work on the synthesis of biologically important Flavonoids compounds and to screen further following scheme.

Encouraged by these reports and in continuation of our interest in the chemistry of 3-flavanols, we have synthesized 2, 3-disubstituted flavan-4-ones by condensation of various chalcone derivatives with hydrogen peroxide in presence of absolute ethanol (95%) in the hope of getting potent pharmacological agents. The present project is aimed at the following,

1. 3-hydroxy flavones is to be synthesized from acetophenone derivatives and corresponding aldehydes by Algar-Flynn-Oyamada reaction.
2. To purify the final compounds by recrystallizing it using appropriate solvents.
3. To identify the compounds by using Solubility, TLC and melting point determination.
4. To characterize all the new compounds by analytical and spectral methods.
5. To evaluate the synthesized compounds for their antihyperglycemic, anti inflammatory and antimicrobial activities.

4. PLAN OF WORK

From the literature review reveals, that the flavanoids have a broad biological and pharmacological activities. The present work was carried out to synthesize and characterize the flavanol derivatives; also to study the pharmacological activity. This work was carried out as outlined below.

1. To synthesize of various chalcone derivatives using acetophenone derivatives as starting materials.
2. To cyclocondense of chalcone derivatives to synthesize flavanoid derivatives.
3. To characterize of synthesized intermediates and compounds quantitively
    a. Solubility data
    b. Melting Point data
    c. TLC analysis data
    d. Elemental analysis data
    e. Spectral studies like IR, NMR and Mass Spectra etc.,
4. Evaluation of Synthesized compounds for the pharmacological activity**.**

5. MATERIALS AND METHODS

All the synthetic work was done by using laboratory grade reagents and solvents. The solvents and reagents were purified and dried according to the procedure given in Vogel's textbook of practical organic chemistry. All the compounds procured were purified dried, whenever necessary before use, following standard methods.

● The compounds were purified by recrystallization using suitable solvents.

● Solubility of all synthesized compounds in different solvents like Water, Ethanol, Acetone, Chloroform, DMSO, and Benzene was determined.

● Melting point of synthesized compounds were determined in open capillary tubes.

● TLC's were performed to monitor the reactors, the reaction and to determine the purity of the products. Thin Layer Chromatography was checked by using silica gel-G coated plates. Iodine vapours are used as visualizing agent.

● IR Spectra were recorded on Shimadzu 8400-S Fourier Transform Infrared Spectrophotometer using Potassium Bromide. (Vmax in cm^{-1}).

● ^{1}H NMR spectra was recorded on Bruker NMR spectrophotometer using Acetone as solvent at Sri Ramachandra University, Chennai and chemical shifts are given in parts per million.

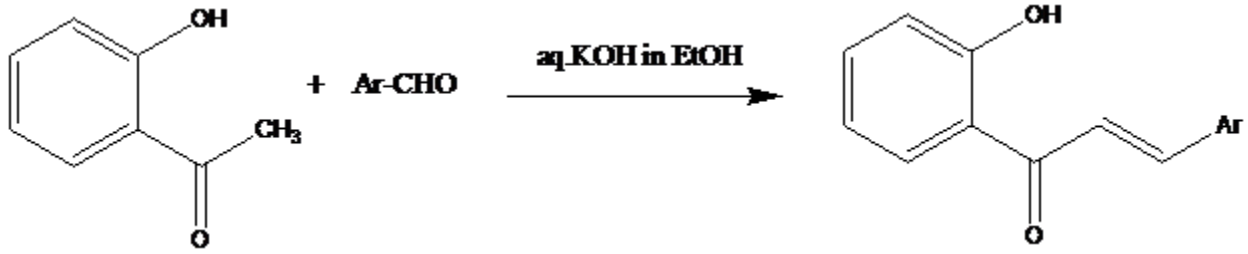
● Mass Spectra were recorded at Sophisticated Analytical Instruments Facility at Indian Institute of Technology, Chennai.

Animal approval

The study was conducted after obtaining the approval from Institutional animal ethics committee (IAEC), and the experimental procedures were in accordance to the guidelines of IAEC (688/02/c/CPCSEA).

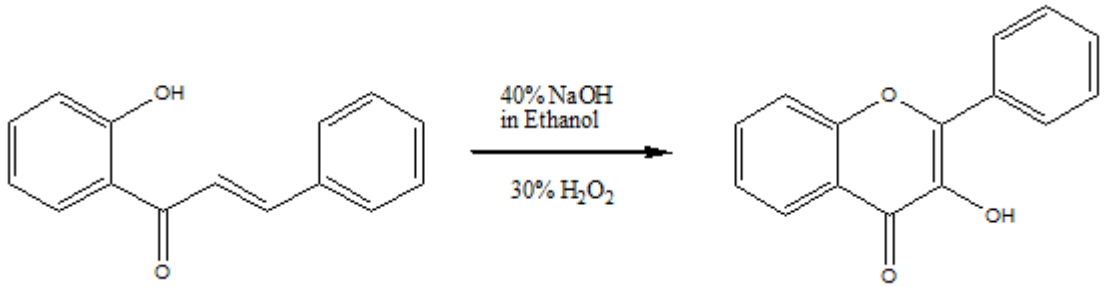
6.1 Scheme of the Work

Step 1 :-



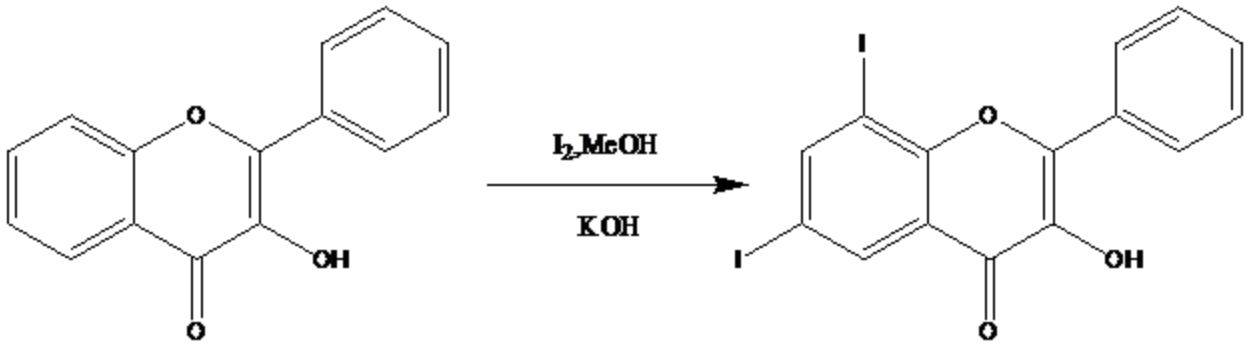
(2 - Hydroxy Acetophenone) (Aromatic Aldehyde) (2-Hydroxy Chalcone Derivative)

Step 2: –



(2-Hydroxy Chalcone Derivative) (2-Phenyl-3-Hydroxy-4H-Chroman-4-one)

Step 3:–



(2-Phenyl-3-Hydroxy-4H-Chroman-4-one) (2-Phenyl-3-Hydroxy-6,8-Diiodo-4H- Chroman-4-one)

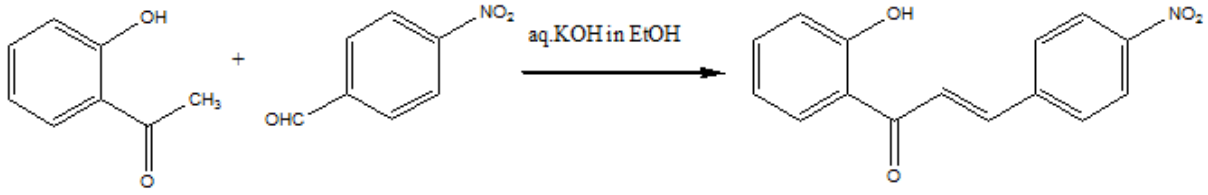
6.2 SYNTHESIS OF COMPOUNDS

COMPOUND A – Synthesis of 2-(4'-nitrophenyl)-3-hydroxy-4H-Chromen-4-one

STEP 1 : Preparation of Chalcone Derivative

Synthesis of 3-(4'-nitrophenyl)-1-(2-hydroxyphenyl)-prop-2-ene-1-one :

A mixture of 2-hydroxy Acetophenone (0.01) and p-nitro benzaldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analysed.

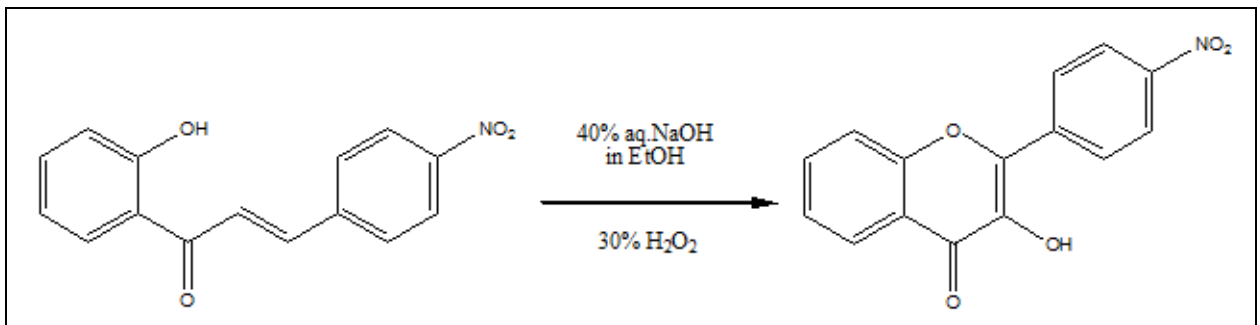


(2-Hydroxy Acetophenone)(4-Nitrobenzaldehyde) (2-Hydroxy Chalcone Derivative)

STEP 2 : Cylisation of Chalcone Derivative and Addition of Hydroxyl group :

Synthesis of 2-(4'-nitrophenyl)-3-hydroxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.



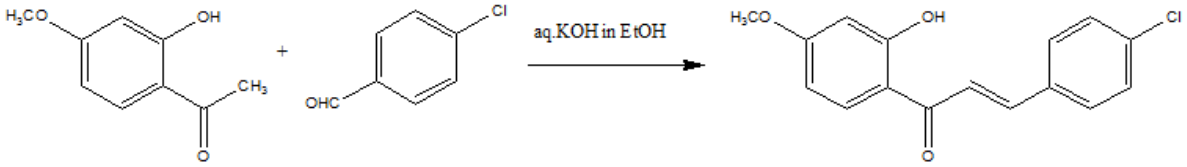
(2-Hydroxy Chalcone Derivative) 2-(4'-nitrophenyl)-3-hydroxy-4H-Chromen-4-one

COMPOUND B – Synthesis of 2-(4-chlorophenyl)-7-methoxy-3-hydroxy-4H-Chromen-4-one

STEP 1: Preparation of Chalcone Derivative

Synthesis of 3-(4'-chlorophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-ene-1-one :

A mixture of 2-hydroxy-4-methoxy Acetophenone (0.01) and p-chloro benzaldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analysed.

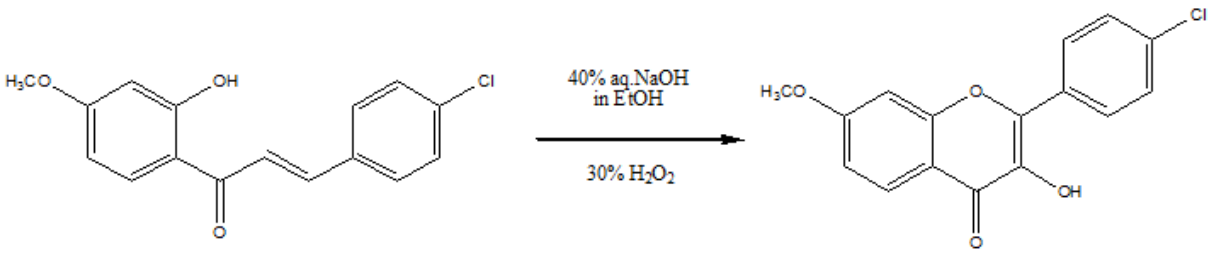


(2-Hydroxy-4-Methoxy Acetophenone) (4-Chlorobenzaldehyde) (2-hydroxy-4-Methoxy Chalcone Drv.)

STEP 2: Cylisation of Chalcone Derivative and Addition of Hydroxyl group:

Synthesis of 2-(4'-chlorophenyl)- 3-hydroxy-7-methoxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.



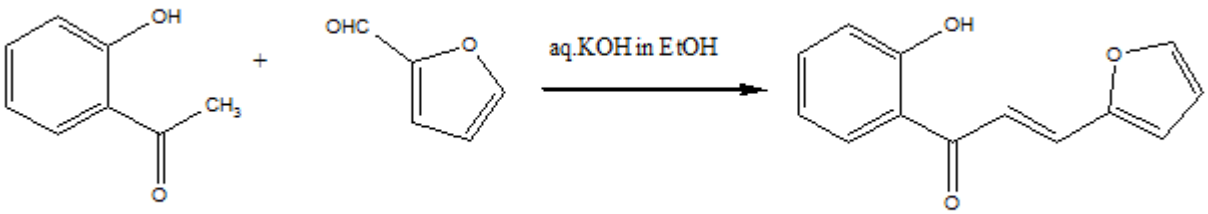
(2-Hydroxy-4-Methoxy Chalcone Drv.) 2-(4-chlorophenyl)-7-methoxy-3-hydroxy 4H-Chromen-4-one

COMPOUND C – Synthesis of 2-(2'-furfural)-3-hydroxy-4H-Chromen-4-one

STEP 1: Preparation of Chalcone Derivative

Synthesis of 3-(2'-furfural)-1-(2-hydroxyphenyl)-prop-2-ene-1-one :

A mixture of 2-hydroxy Acetophenone (0.01) and Furfuraldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analysed.

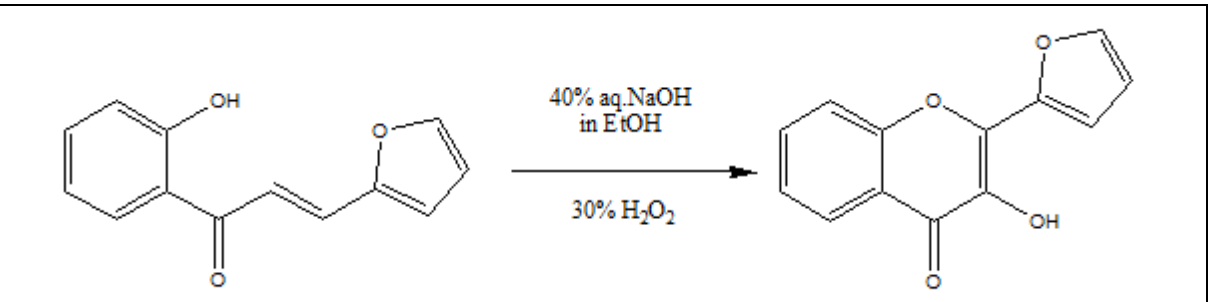


(2-Hydroxy Acetophenone) (Furfuraldehyde) (2-Hydroxy Furfural Chalcone Drv.)

STEP 2: Cylisation of Chalcone Derivative and Addition of Hydroxyl group :

Synthesis of 2-(2'-furfural)- 3-hydroxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.



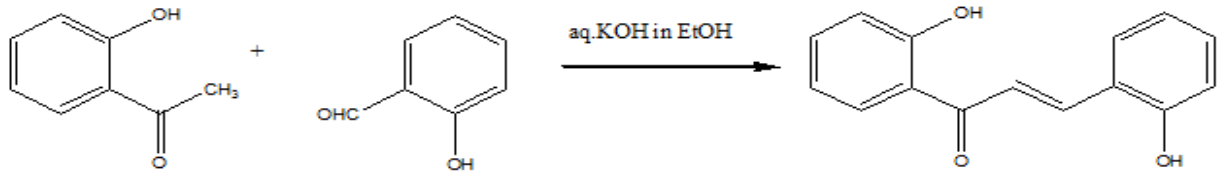
(2-Hydroxy Furfural Chalcone Drv.) 2-(2'-fufural)-3-hydroxy-4H-Chromen-4-one)

COMPOUND D – Synthesis of 2-(2'-hydroxyphenyl)-3-hydroxy-4H-Chromen-4-one

STEP 1: Preparation of Chalcone Derivative

Synthesis of 3-(2'-hydroxyphenyl)-1-(2-hydroxyphenyl)-prop-2-ene-1-one :

A mixture of 2-hydroxy Acetophenone (0.01) and Salicylaldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralized with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analyzed.

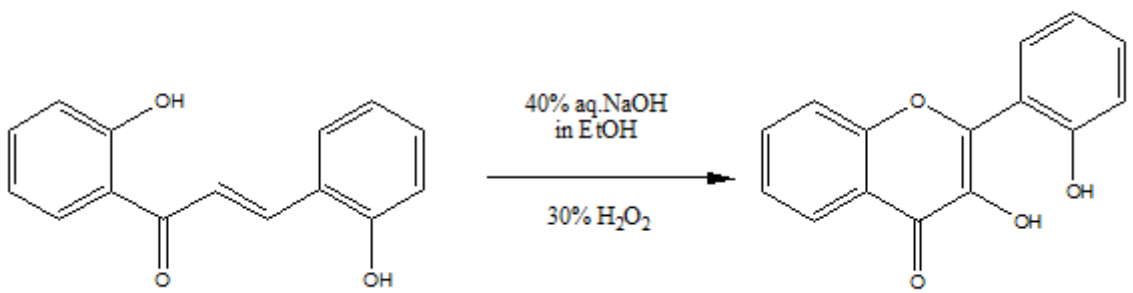


(2-Hydroxy Acetophenone) (2-Hydroxy benzaldehyde) (2-Hydroxy Chalcone Derivative)

STEP 2 : Cylisation of Chalcone Derivative and Addition of Hydroxyl group :

Synthesis of 2-(2'-hydroxyphenyl)- 3-hydroxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.



(2-Hydroxy Chalcone Derivative) 2-(2'-hydroxyphenyl)-3-hydroxy-4H-

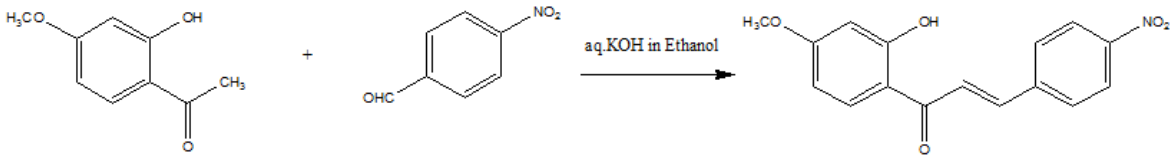
Chromen-4-one

COMPOUND E – Synthesis of 2-(4'-nitrophenyl)-7-methoxy-3-hydroxy-4H-Chromen-4-one

STEP 1: Preparation of Chalcone Derivative

Synthesis of 3-(4'-nitrophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-ene-1-one:

A mixture of 2-hydroxy-4-methoxy Acetophenone (0.01) and p-nitro benzaldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analysed.

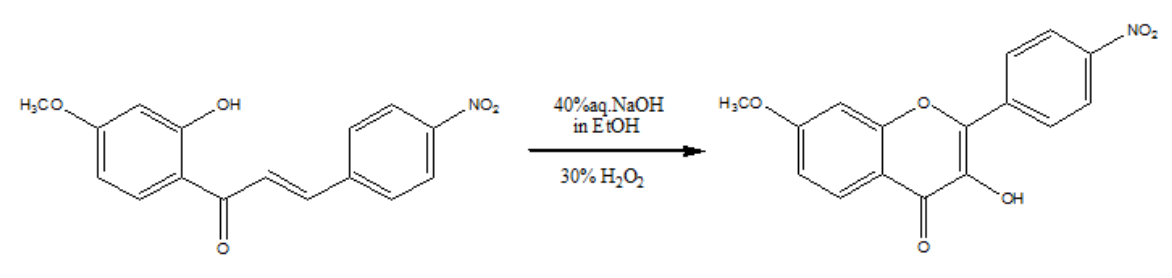


(2-Hydroxy-4-Methoxy Acetophenone) (4-Nitrobenzaldehyde) (2-Hydroxy-4-Methoxy Chalcone Drv.)

STEP 2 : Cylisation of Chalcone Derivative and Addition of Hydroxyl group :

Synthesis of 2-(4'-nitrophenyl)- 3-hydroxy-7-methoxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.



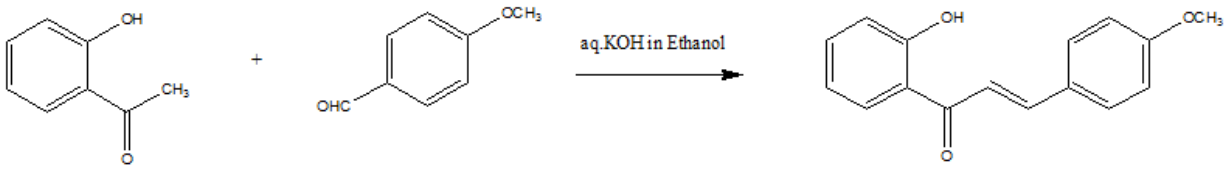
(2-Hydroxy-4-Methoxy Chalcone Drv.) 2-(4'-nitrophenyl)-3-hydroxy-7-methoxy Chromen-4-one

COMPOUND F – Synthesis of 2-(4'-methoxyphenyl)-3-hydroxy-4H-Chromen-4-one

STEP 1: Preparation of Chalcone Derivative

Synthesis of 3-(4'-methoxyphenyl)-1-(2-hydroxyphenyl)-prop-2-ene-1-one:

A mixture of 2-hydroxy Acetophenone (0.01) Anisaldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analysed.

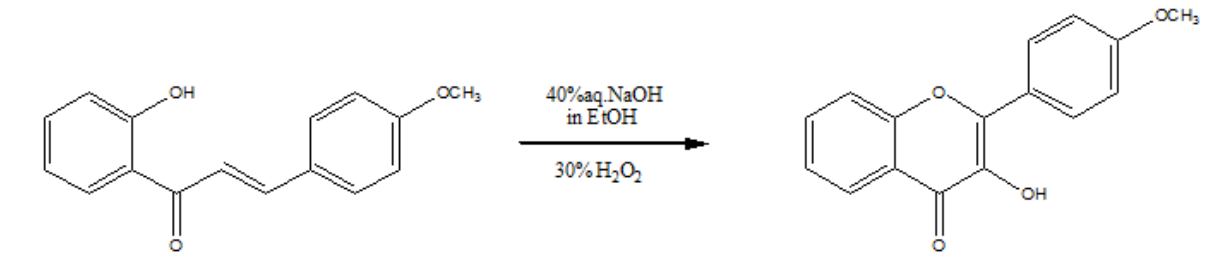


(2-Hydroxy Acetophenone) (4-Methoxy benzaldehyde) (Hydroxy Chalcone Derivative)

STEP 2 : Cylisation of Chalcone Derivative and Addition of Hydroxyl group :

Synthesis of 2-(4'-methoxyphenyl)-3-hydroxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.



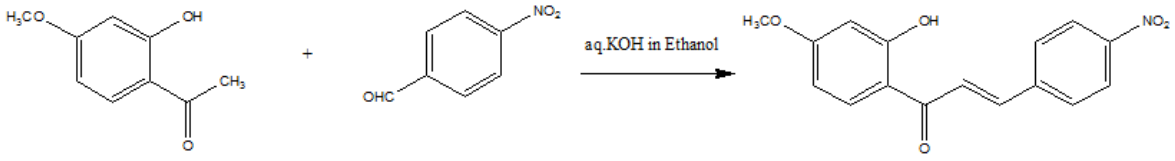
(Hydroxy Chalcone Derivative) 2-(4-methoxyphenyl)-3-hydroxy-4H-Chromen-4-one

COMPOUND G – Synthesis of 2-(4-nitrophenyl)-6,8-diiodo-3-hydroxy-7-methoxy-4H-Chromen-4-one

STEP 1: Preparation of Chalcone Derivative

Synthesis of 3-(4'-nitrophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-ene-1-one :

A mixture of 2-hydroxy-4-methoxy Acetophenone (0.01) and p-nitro benzaldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analysed.

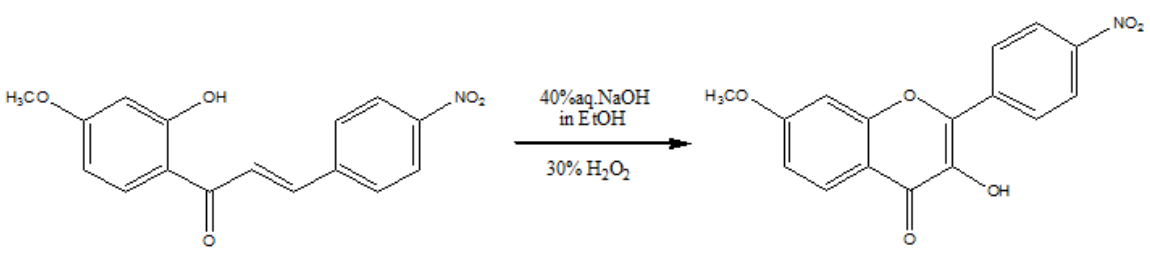


(2-Hydroxy-4-Methoxy Acetophenone) (4-Nitrobenzaldehyde) (2-Hydroxy-4-Methoxy Chalcone Drv.)

STEP 2 : Cylisation of Chalcone Derivative and Addition of Hydroxyl group :

Synthesis of 2-(4'-nitrophenyl)- 3-hydroxy-7-methoxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.

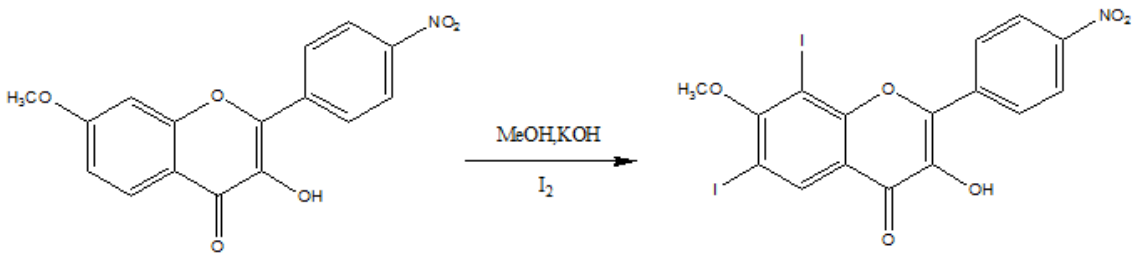


(2-Hydroxy-4-Methoxy Chalcone Drv.) 2-(4'-nitrophenyl)-3-hydroxy-7-methoxy Chromen-4-one

STEP-3: Addition of Iodine :

Synthesis of 2-(4'-nitrophenyl)-3-hydroxy-6,8-diiodo-7-methoxy-4H-Chromen-4- one :

The final product is dissolved in 10ml of Methanol,add Potassium Hydroxide(25mg) with stirring and add Iodine. Keep solution for room temperature, stir for 3 hrs. Evaporate the solution to obtain the product.



2-(4'-nitrophenyl)-3-hydroxy-7-methoxy Chromen-4-one

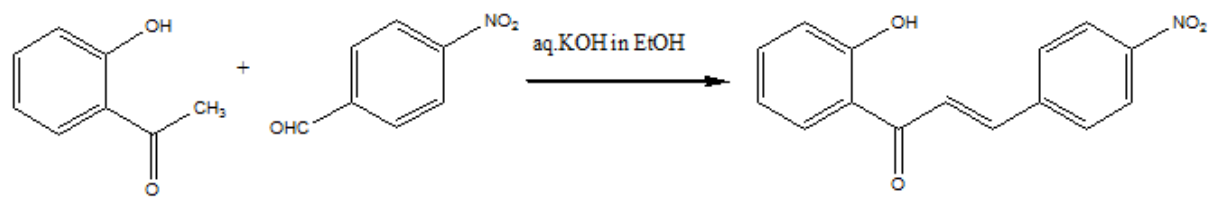
2-(4'-nitrophenyl)-3-hydroxy-6,8-diiodo-7-methoxy-4H-Chromen-4-one

COMPOUND H – Synthesis of 2-(4'-nitrophenyl)-3-hydroxy-6,8-diiodo-4H-Chromen-4-one

STEP 1 : Preparation of Chalcone Derivative

Synthesis of 3-(4'-nitrophenyl)-1-(2-hydroxyphenyl)-prop-2-ene-1-one :

A mixture of 2-hydroxy Acetophenone (0.01) and p-nitro benzaldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analysed.



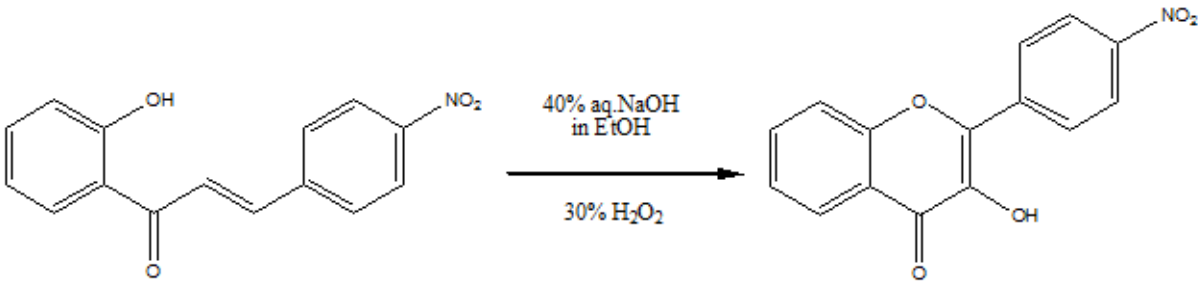
(2-Hydroxy Acetophenone)(4-Nitrobenzaldehyde)

(2-Hydroxy Chalcone Derivative)

STEP 2 : Cylisation of Chalcone Derivative and Addition of Hydroxyl group :

Synthesis of 2-(4'-nitrophenyl)-3-hydroxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.

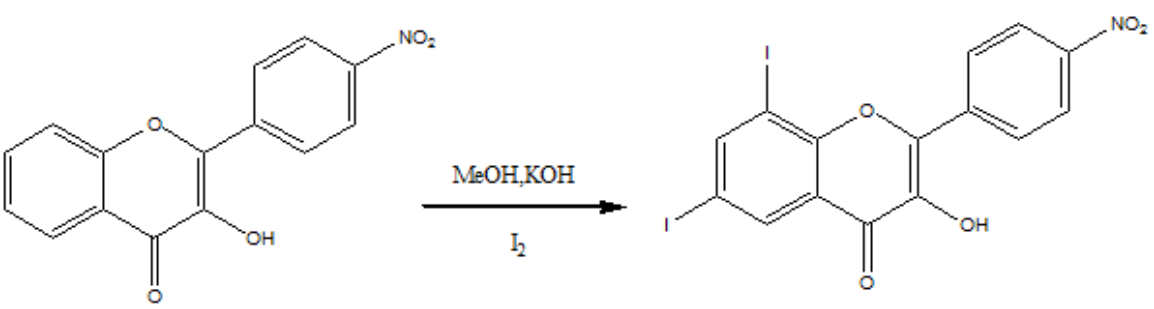


(2-Hydroxy Chalcone Derivative) 2-(4'-nitrophenyl)-3-hydroxy-4H-Chromen-4-one

STEP-3 : Addition of Iodine :

Synthesis of 2-(4'-nitrophenyl)-3-hydroxy-6,8-Diiodo-4H-Chromen-4-one :

The final product is dissolved in 10ml of Methanol,add Potassium Hydroxide(25mg) with stirring and add Iodine. Keep solution for room temperature, stir for 3 hrs. Evaporate the solution to obtain the product.



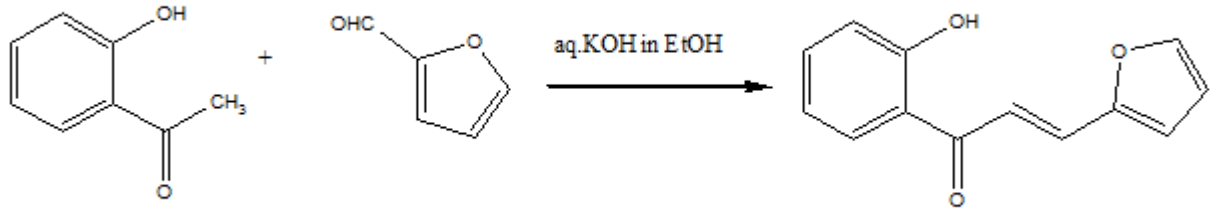
2-(4'-nitrophenyl)-3-hydroxy-4H-Chromen-4-one 2-(4'-nitrophenyl)-3-hydroxy-6,8-diiodo 4H-Chromen- 4-one

COMPOUND I – Synthesis of 2-(2'-furfural)-3-hydroxy-6,8-diiodo-4H-Chromen-4-one

STEP 1: Preparation of Chalcone Derivative

Synthesis of 3-(2'-furfural)-1-(2-hydroxyphenyl)-prop-2-ene-1-one:

A mixture of 2-hydroxy Acetophenone (0.01) and Furfuraldehyde (0.01) was stirred in 30 ml of absolute ethanol (95%) and then aqueous solution of potassium hydroxide was added to it. The reaction mixture was kept overnight at room temperature and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The chalcone derivative precipitates out as solid that was filtered dried and recrystallised from ethanol and analysed.

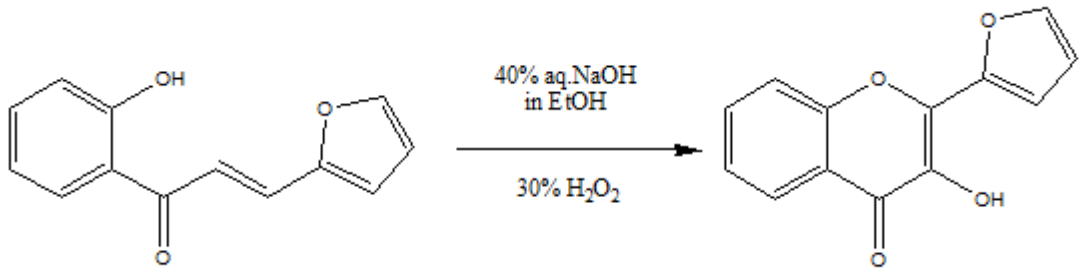


(2-Hydroxy Acetophenone) (Furfuraldehyde) (2-Hydroxy Furfural Chalcone Drv.)

STEP 2 : Cylisation of Chalcone Derivative and Addition of Hydroxyl group :

Synthesis of 2-(2'-furfural)- 3-hydroxy-4H-Chromen-4-one :

The intermediate, add 10 ml of hydrogen peroxide drop wise maintaining temperature 10 ^{o}C, stir for 2 hrs and then poured on crushed ice. The mixture was then neutralised with dil.HCl acid. The solid product was filtered, washed with ice cold water.

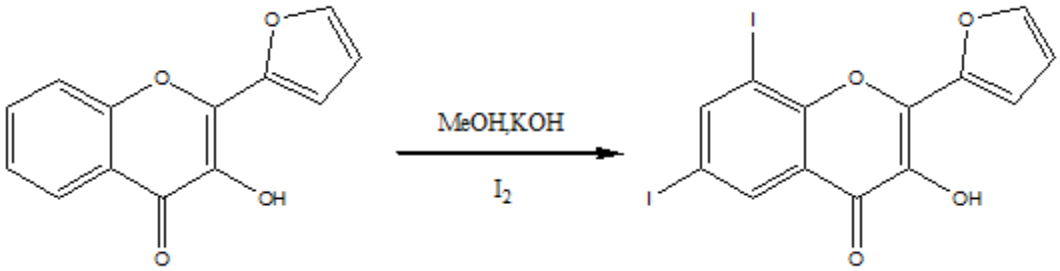


(2-Hydroxy Furfural Chalcone Drv.) 2-(2'-fufural)-3-hydroxy-4H-Chromen-4-one)

STEP-3: Addition of Iodine:

Synthesis of 2-(4'-nitrophenyl)-3-hydroxy-6,8-Diiodo-4H-Chromen-4-one :

The final product is dissolved in 10ml of Methanol,add Potassium Hydroxide(25mg) with stirring and add Iodine. Keep solution for room temperature, stir for 3 hrs. Evaporate the solution to obtain the product.



2-(2'-fufural)-3-hydroxy-4H-Chromen-4-one)

2-(2'-furfural)-3-hydroxy-6,8-diiodo 4H-Chromen-4-one

6.3 SOLUBILITY STUDIES[63, 64]

Table No. 1

S.No	SOLVENT / COMPOUND	Water	Ethanol	Acetone	Chloroform	Benzene	DMSO
1.	Intermediate I	−	±	+++	++	++	+++
2.	Intermediate II	−	±	++	++	++	+++
3.	Intermediate III	−	+	++	++	++	+++
4.	Intermediate IV	−	+	+++	+++	+++	+++
5.	Intermediate V	−	±	+++	+++	+++	+++
6.	Intermediate VI	−	±	+++	+++	+++	+++
7.	Intermediate VII	−	±	++	++	+++	+++
8.	Intermediate VIII	−	±	+++	+++	++	+++
9.	Intermediate IX	−	+	+++	+++	+++	+++

Key: +++, ++, + → Soluble; - → Insoluble; ± → Sparingly soluble

6.4 CHEMICAL CHARACTERIZATION AND SPECTRAL STUDIES FOR INTERMEDIATE

The structure of all the newly synthesized intermediate was elucidated by IR spectra.IR spectra were recorded on Shimadzu FTIR-8400's spectrophotometer. The IR values were measured in $cm^{-1}$and the results are shown below.

1. INTERMEDIATE I: 3-(4-nitrophenyl)-1-(2-hydroxyphenyl)-prop-2-ene-1-one

IR (KBr cm^{-1}) 3634.97 (phenolic O-H stretch), 2978.19 (aromatic C-H stretch), 1686.81 (C=O stretch), 1520.92 (aromatic C=C stretch), 1349.25 (N=O stretch)

2. INTERMEDIATE II: 3-(4-chlorophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-ene-1-one

IR (KBr cm^{-1}) 2980.12 (aromatic C-H stretch), 1591.33 (C=O stretch), 1569.14 (aromatic C=C stretch), 1223.87 (C-O stretch), 789.88 (C-Cl stretch)

3. INTERMEDIATE III: 3-(2'-furfural)-1-(2-hydroxyphenyl)-prop-2-ene-1-one

IR (KBr cm^{-1}) 3614.72 (phenolic O-H stretch), 2994.59 (aromatic C-H stretch), 1642.44 (C=O stretch), 1554.68 (aromatic C=C stretch), 1214.23 (C-O stretch).

4. INTERMEDIATE IV: 3-(2'-hydroxyphenyl)-1-(2-hydroxyphenyl)-prop-2-ene-1-one

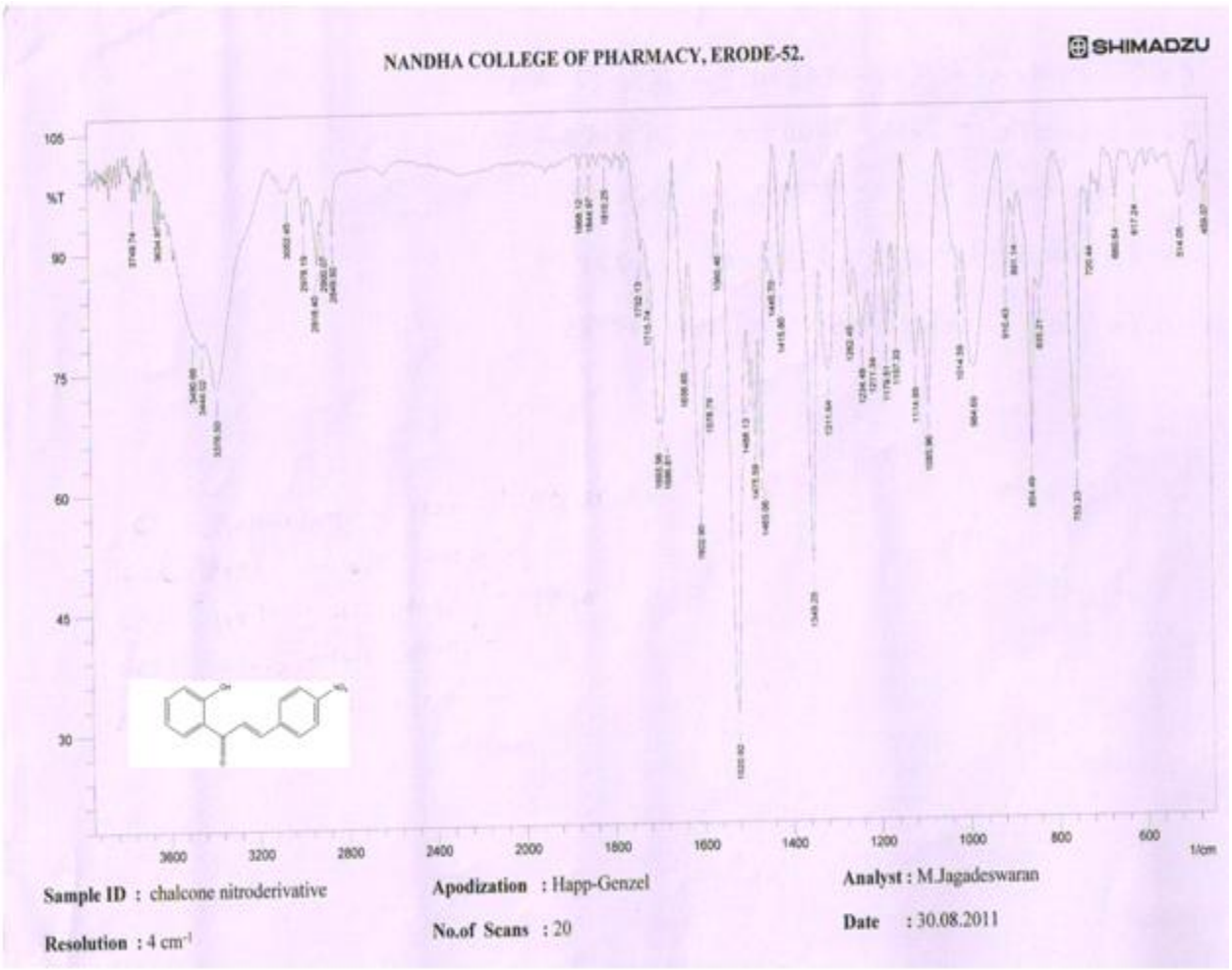
IR(KBr cm^{-1}) 3284.88 (phenolic O-H stretch), 1629.90 (C=O stretch), 1584.57 (aromatic C=C stretch), 1263.42 (C-O stretch).

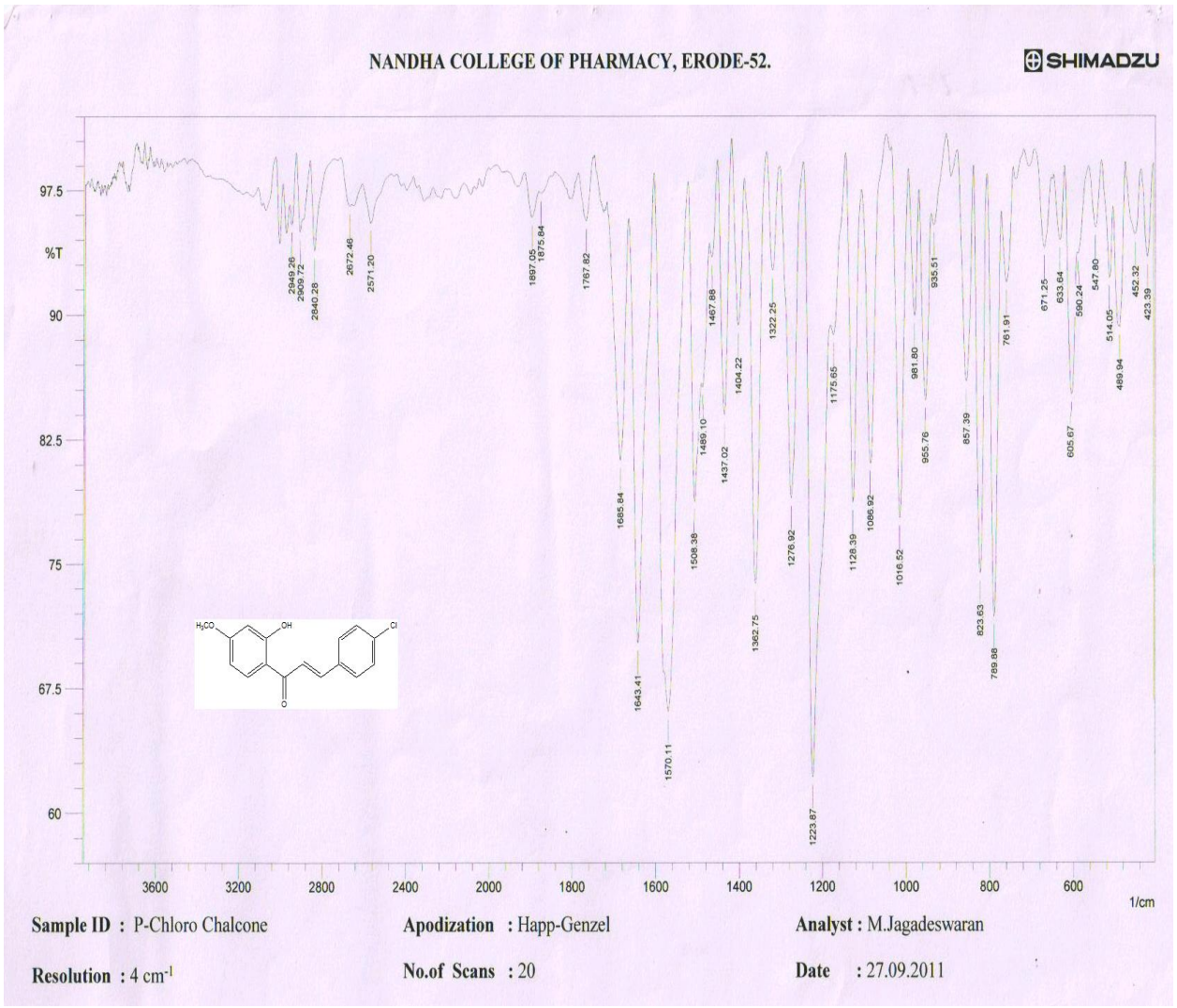
5. INTERMEDIATE V: 3-(4-nitrophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-ene-1-one

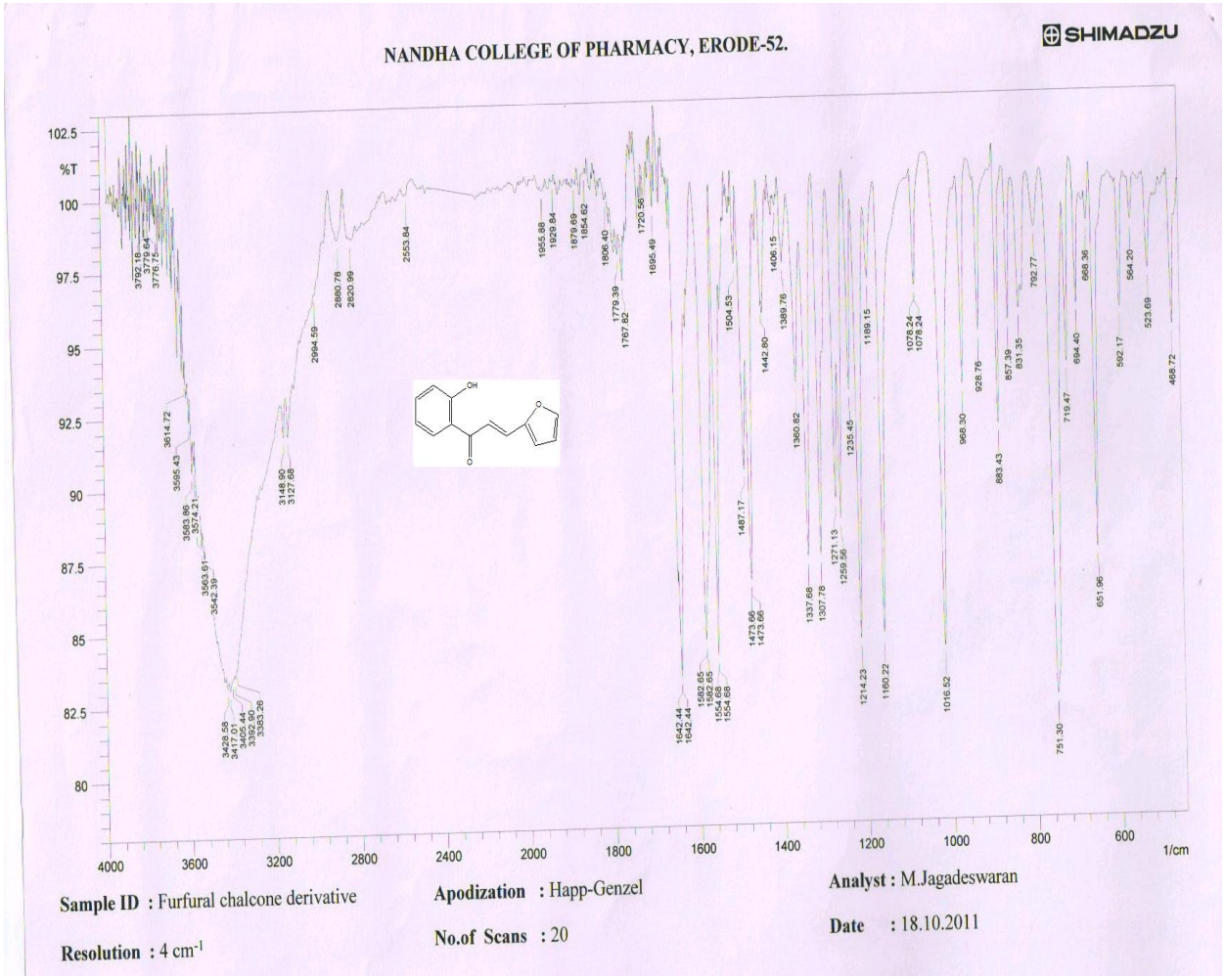
IR (KBr cm^{-1}) 3526.96 (phenolic O-H stretch), 2921.29 (aromatic C-H stretch), 1696.45 (C=O stretch), 1523.82 (aromatic C=C stretch), 1345.39 (N=O stretch), 1106.21 (C-O stretch).

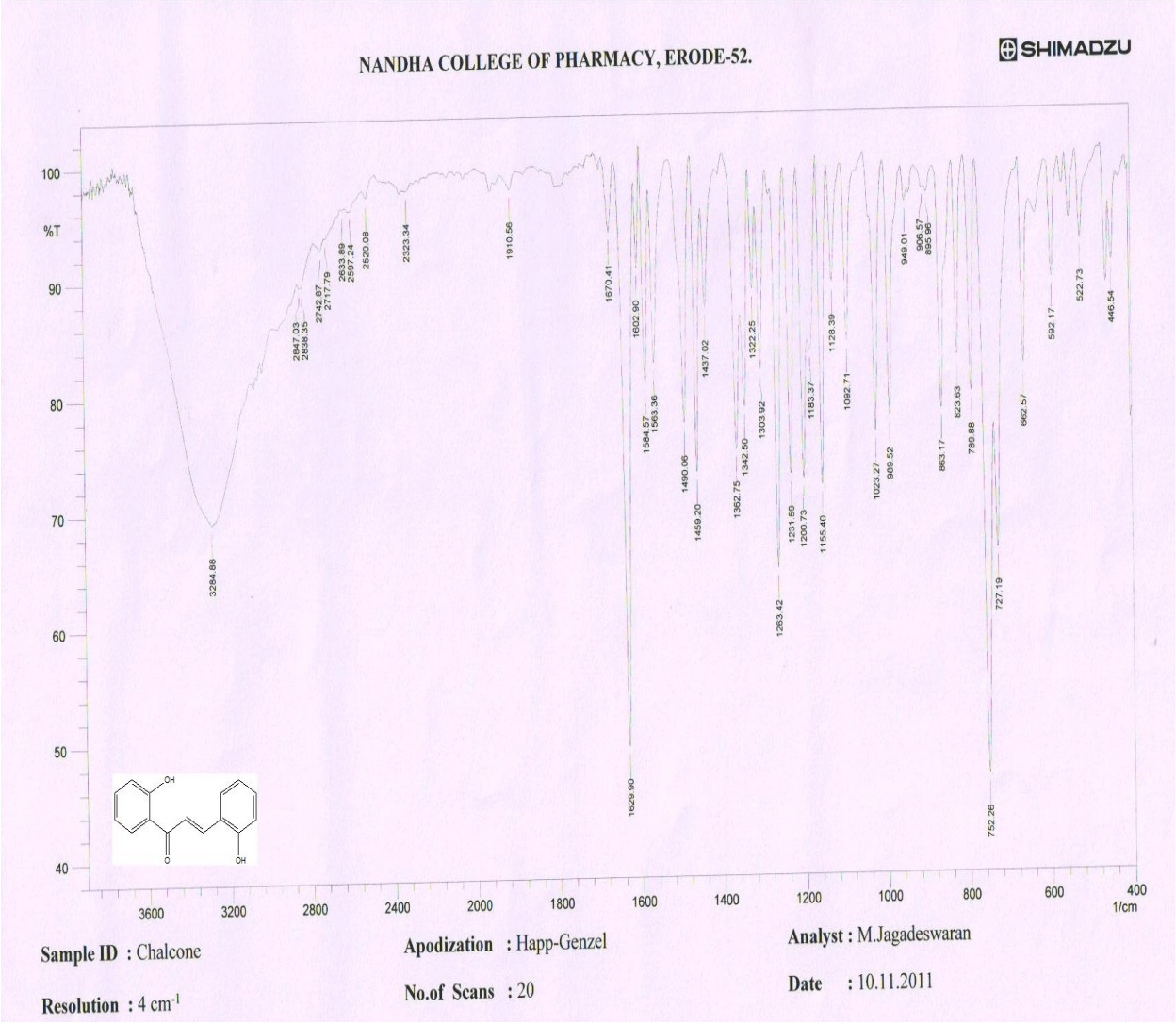
6. INTERMEDIATE VI: 3-(4-methoxyphenyl)-1-(2-hydroxyphenyl)-prop-2-ene-1-one

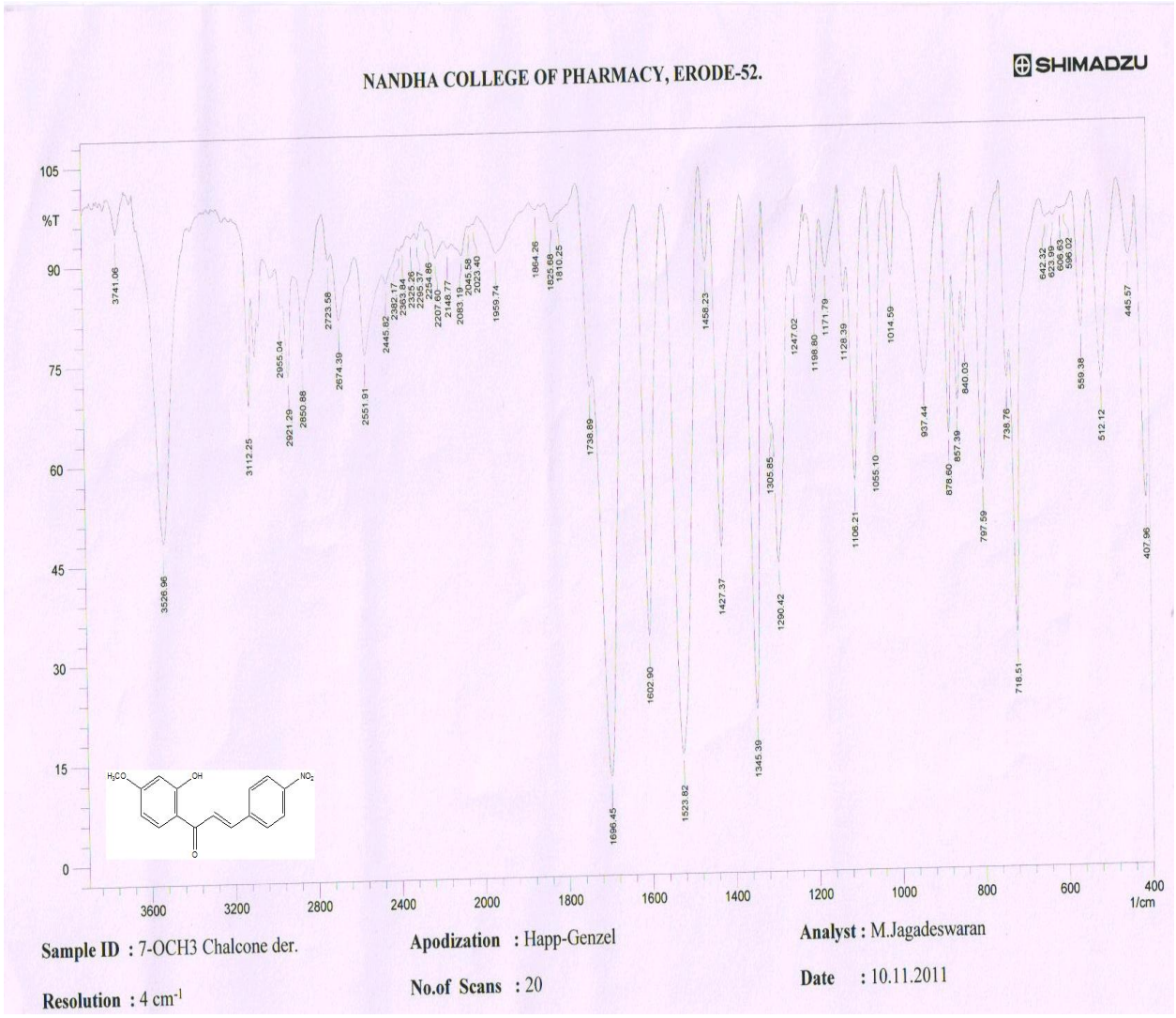
IR (KBr cm^{-1}) 3563.61 (phenolic O-H stretch), 3025.45 (aromatic C-H stretch), 2913.57 (alkane C-H stretch), 1639.36 (C=O stretch), 1563.36(aromatic C=C stretch), 1030.02 (C-O stretch)

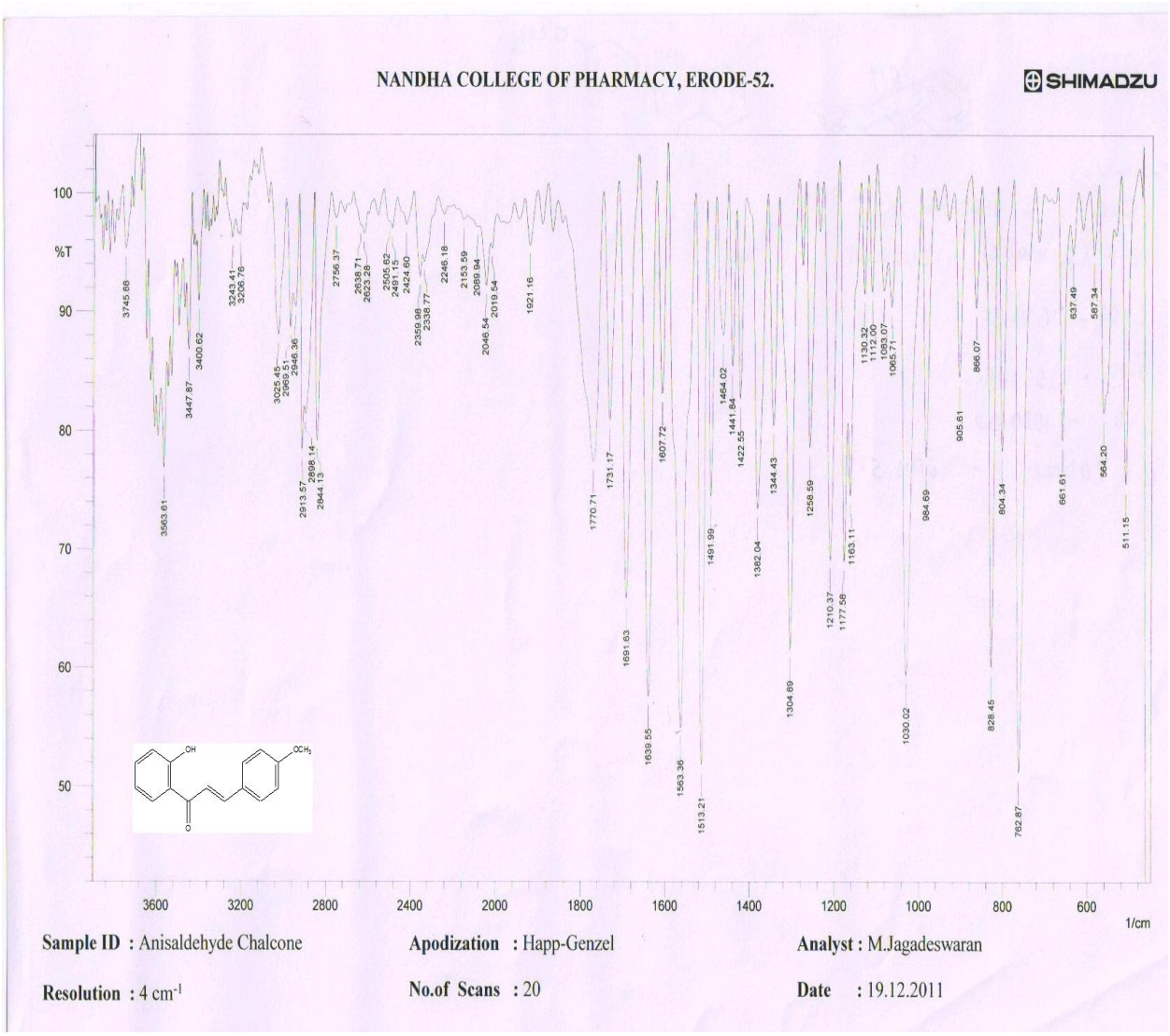

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
105
90
75
60
45
30
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
1/cm
Sample ID : chalcone nitroderivative
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 30.08.2011


NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
97.5
%T
90
82.5
75
67.5
60
2949.26
2909.72
2840.28
2672.46
2571.20
1897.05
1875.84
1767.82
1685.84
1643.41
1570.11
1508.38
1489.10
1467.88
1437.02
1404.22
1362.75
1322.25
1276.92
1223.87
1175.65
1128.39
1086.92
1016.52
981.80
955.76
935.51
857.39
823.63
789.88
761.91
671.25
633.64
605.67
590.24
547.80
514.05
489.94
452.32
423.39
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
1/cm
Sample ID : P-Chloro Chalcone
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 27.09.2011


NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
1/cm
Sample ID : Furfural chalcone derivative
Resolution : 4 cm-1
Apodization : Happ-Genzel
No.of Scans : 20
Analyst : M.Jagadeswaran
Date : 18.10.2011


NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
100
%T
90
80
70
60
50
40
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
3284.88
2847.03
2838.35
2748.87
2717.79
2633.89
2597.24
2520.08
2323.34
1910.56
1670.41
1629.90
1602.90
1584.57
1563.36
1490.06
1459.20
1437.02
1362.75
1342.50
1322.25
1303.92
1263.42
1231.59
1200.73
1183.37
1155.40
1128.39
1082.71
1023.27
989.52
949.01
906.57
895.96
863.17
823.63
789.88
752.26
727.19
662.57
592.17
522.73
446.54
OH
O
OH
Sample ID : Chalcone
Resolution : 4 cm-1
Apodization : Happ-Genzel
No.of Scans : 20
Analyst : M.Jagadeswaran
Date : 10.11.2011


NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
1/cm
Sample ID : 7-OCH3 Chalcone der.
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 10.11.2011


NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
1/cm
Sample ID : Anisaldehyde Chalcone
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 19.12.2011

6.5 SOLUBILITY STUDIES[63,64]

Table No. 2

S.No	SOLVENT / COMPOUND	Water	Ethanol	Acetone	Chloroform	Benzene	DMSO
1.	Compound I	–	±	+++	+++	++	+++
2.	Compound II	–	+	+++	++	++	+++
3.	Compound III	–	+	+++	+++	+++	+++
4.	Compound IV	–	±	+++	++	+++	+++
5.	Compound V	–	±	++	++	+++	+++
6.	Compound VI	–	+	+++	++	++	+++
7.	Compound VII	–	±	+++	+++	++	+++
8.	Compound VIII	–	±	+++	++	+++	+++
9.	Compound IX	–	+	++	+++	+++	+++

Key: +++, ++, + → Soluble; - → Insoluble ; ± → Sparingly soluble

6.6 CHARACTERISATION DATA FOR THE SYNTHESISED COMPOUNDS

Table No. 3

Sr. No.	Compounds	Mol. Formula	Mol. Weight	M.P (^{o}C)	R_f Value	% Yield (w/w)	Elemental Analysis Calculated (%)					
							C	H	O	N	Cl	I
1	A	$C_{15}H_9O_5N$	283.23	126-128	0.38	83.67	63.61	3.21	28.24	4.94	_	_
2	B	$C_{16}H_{11}O_4Cl$	302.71	168-170	0.803	72.43	63.48	3.66	21.14	_	11.72	_
3	C	$C_{13}H_8O_4$	228.2	130-132	0.508	72.92	68.43	3.53	28.04	_	_	_
4	D	$C_{15}H_{10}O_4$	254.24	136-138	0.396	67.22	70.86	3.96	25.18	_	_	_
5	E	$C_{16}H_{11}O_6N$	313.26	146-148	0.649	77.27	61.34	3.54	30.64	4.48	_	_
6	F	$C_{16}H_{12}O_4$	268.26	128-130	0.532	52.97	71.63	4.51	23.86	_	_	_
7	G	$C_{16}H_9O_6NI_2$	565.04	140-142	0.54	53.2	34.02	1.61	16.99	2.47	_	44.91
8	H	$C_{15}H_7O_5NI_2$	535.02	132-134	0.542	58.62	33.68	1.32	14.96	2.61	_	47.43
9	I	$C_{13}H_6O_4I_2$	479.98	138-140	0.448	67.02	32.53	1.26	13.34	_	_	52.87

Mobile Phase: Chloroform: Ethyl Acetate: Formic acid (5:4:1)

6.7 SPECTRAL CHARACTERIZATION FOR SYNTHESIZED COMPOUNDS

Table No. 4

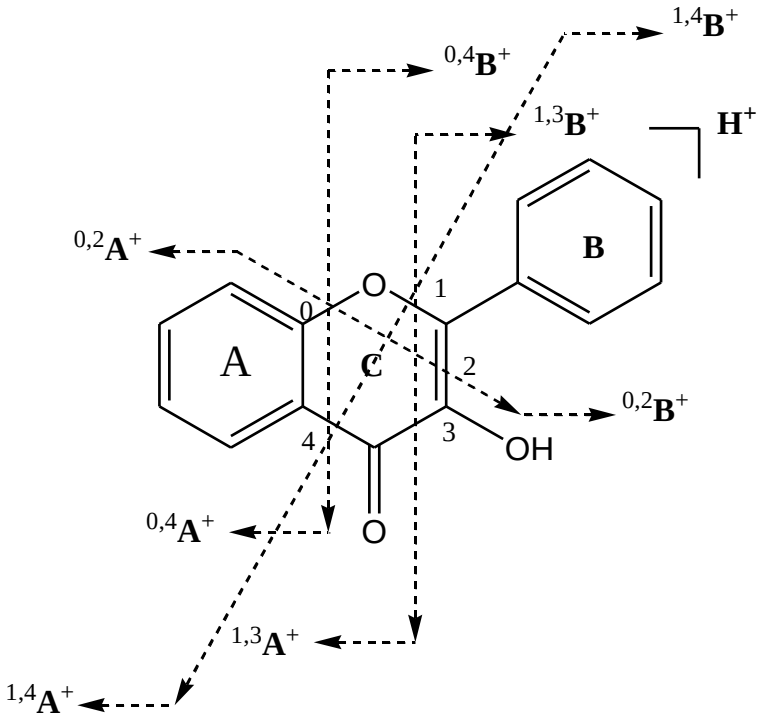
SR.NO	COMPOUNDS	IR(KBr) cm^{-1}	^{1}HNMR(δppm)	Mass m/z
1.	A	3524.06 (phenolic O-H stretch), 2979.16 (aromatic C-H stretch), 1696.46 (C=O stretch), 1521.89 (aromatic C=C stretch), 1349.25 (N=O stretch).	9.704(OH, enol, s), 7.027-8.308(8H, Benzene, m).	282.8531(M^+), 268.4701, 234.3168, 147.9133, 136.0469($^{0,2}A^+$), 164.8477 ($^{1,3}B^+$), 258.4454($^{1,3}A^+$), 82.5106($^{0,4}A^+$).
2.	B	2980.12 (aromatic C-H stretch), 1591.33 (C=O stretch), 1569.14 (aromatic C=C stretch),1088.85 (C-O stretch), 759.01 (C-Cl stretch).	9.572(OH, enol, s), 6.325-8.176(7H, Benzene, m), 3.880(OCH_3, s).	302.0135(M^+), 281.2148, 252.0160, 219.0058, 149.0659, 118.0014($^{1,3}B^+$), 129.0771($^{0,2}A^+$), 209.0679($^{1,3}A^+$), 82.9453($^{0,4}A^+$), 166.0768(M+H-B ring).
3.	C	3634.97 (phenolic O-H stretch), 3079.46 (aromatic C-H stretch), 1668.48 (C=O stretch), 1556.61 (aromatic C=C stretch), 1203.62 (C-O stretch).	9.543(OH, enol, s), 6.981-7.938(4H, Benzene, m), 4.025(3H, Furan, m).	228.1000(M^+), 212.1622, 152.4382, 200.0538($^{1,3}A^+$), 133.4982($^{0,2}A^+$), 112.4514($^{1,3}B^+$), 78.5638($^{0,4}A^+$), 66.5681(M+H-A ring)

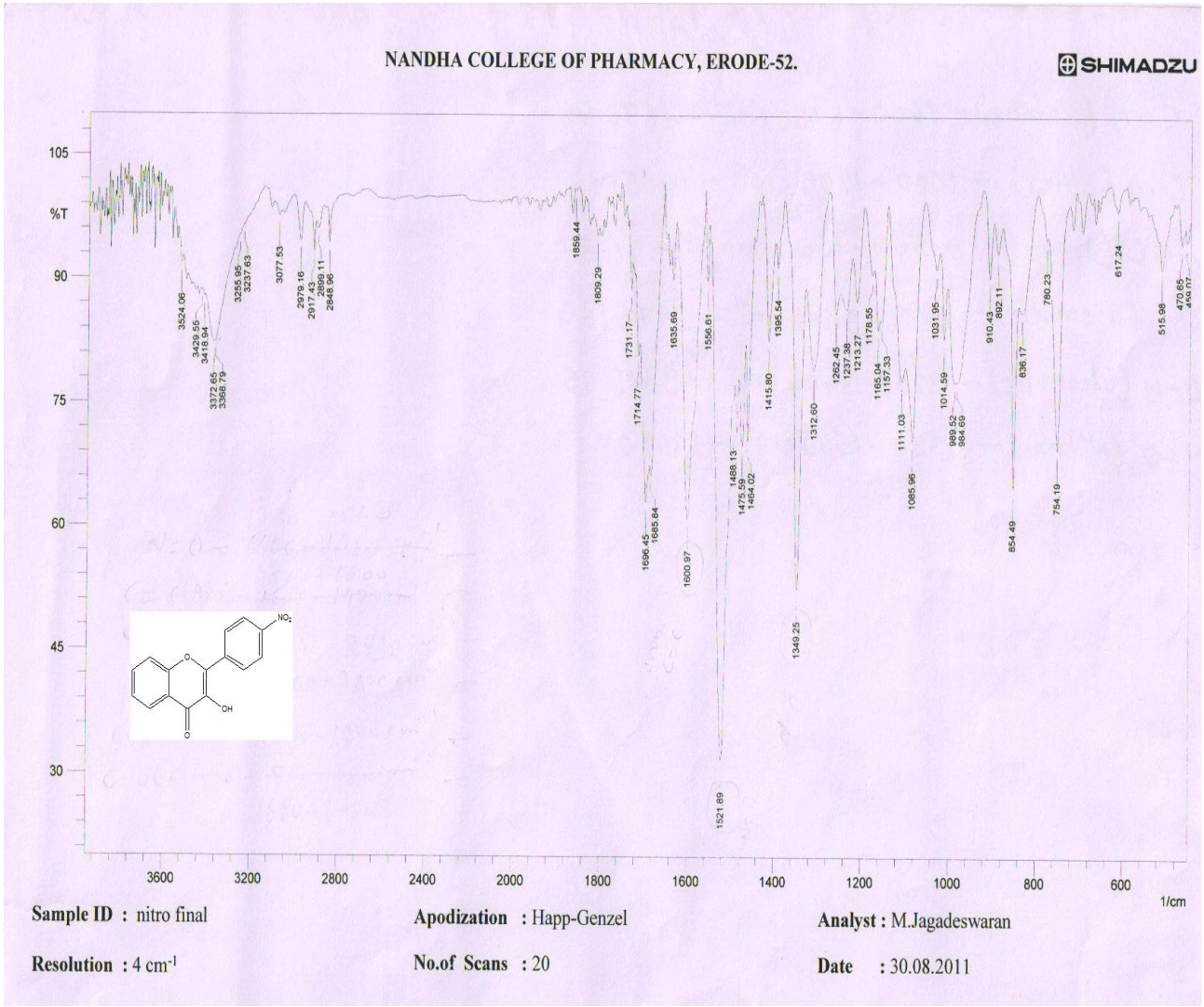
SR.NO	COMPOUNDS	IR(KBr) cm^{-1}	^{1}HNMR(δppm)	Mass m/z
4.	D	3296.46 (phenolic O-H stretch), 2956.57 (aromatic C-H stretch), 1608.69 (C=O stretch), 1563.36 (aromatic C=C stretch), 1285.60 (C-O stretch).	9.702(OH, enol, s), 6.947-8.208(8H, Benzene, m).	255.1000(M^+), 238.1801, 222.2249, 149.4613, 122.5342($^{1,3}B^+$), 135.5544($^{0,2}A^+$), 79.6516($^{0,4}A^+$), 162.489(M=H-B ring).
5.	E	3525.99 (phenolic O-H stretch), 1695.49 (C=O stretch), 1524.78 (aromatic C=C stretch), 1348.39 (N=O stretch), 1106.21 (C-O stretch).	9.638(OH, enol, s), 6.672-8.382(7H, Benzene, m), 3.496(OCH_3, s).	307.0275(M^+), 296.5642, 252.0160, 220.8764, 147.2218, 118.0014($^{1,3}B^+$), 136.7824($^{0,2}A^+$), 78.6532($^{0,4}A^+$), 164.2356(M=H-B ring).
6.	F	3644.62 (phenolic O-H stretch), 3214.48 (aromatic C-H stretch), 2950.22 (alkane C-h stretch), 1639.55 (C=O stretch), 1605.79 (aromatic C=C stretch), 1028.09 (C-O stretch).	12.733(OH, enol, s), 6.915-8.266(8H, Benzene, m), 3.869(OCH_3, s).	268.9920(M^+), 249.3303, 220.2907, 146.9495, 135.2109($^{0,2}A^+$), 118.7018($^{1,3}B^+$), 211.5220($^{1,3}A^+$), 79.6628(M+H-B ring).

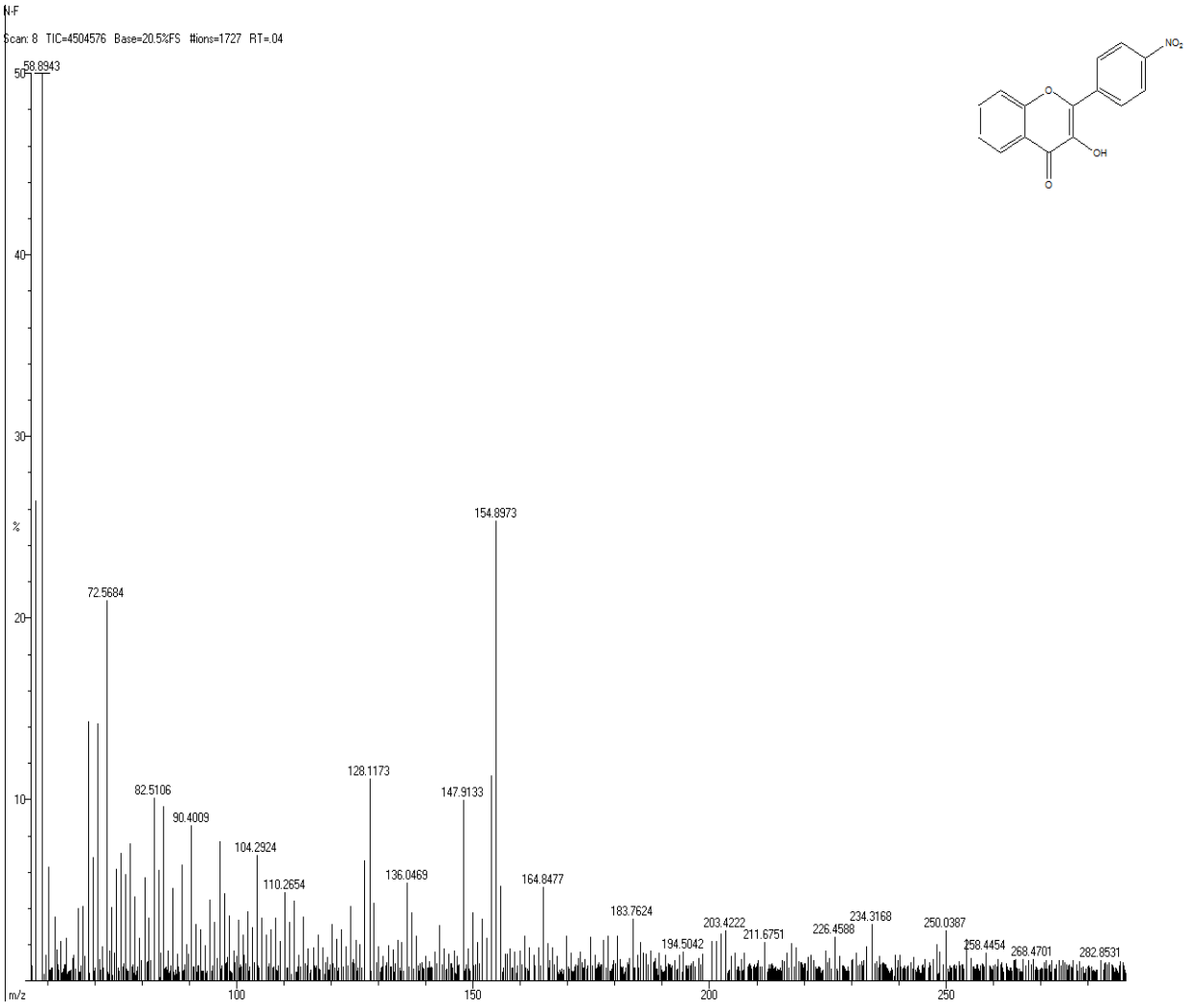
SR.NO	COMPOUNDS	IR(KBr) cm^{-1}	^{1}HNMR(δppm)	Mass m/z
7.	G	3541.42 (phenolic O-H stretch), 2997.48 (aromatic C-H stretch), 1693.56 (C=O stretch), 1524.78 (aromatic C=C stretch), 1346.36 (N=O stretch), 1107.18 (C-O stretch), 719.47 (C-I stretch).	9.542(OH, enol, s), 6.281-8.384(6H, Benzene, m).	563.5431(M^+), 547.3876, 501.5643, 249.4911, 220.5648, 147.2854, 123.5311($^{1,3}B^+$), 278.5090($^{1,3}A^+$), 81.8404($^{0,4}A^+$), 164.4571(M+H-B ring).
8.	H	3610.86 (phenolic O-H stretch),2982.05 (aromatic C-H),1773.61 (aromatic C=C stretch), 1636.19 (aromatic C=O stretch), 1520.92 (N=O stretch), 1092.71 (C-O stretch), 755.16 (C-I stretch).	9.668(OH, enol, s), 8.269-8.387(5H, Benzene, m).	543.1000(M^+), 517.5698, 468.5626, 220.8973, 149.7674, 120.0275($^{1,3}B^+$), 388.2298($^{0,2}A^+$), 207.4306($^{1,3}A^+$), 84.3313($^{0,4}A^+$), 160.3678(M+H_B ring).
9.	I	3454.62 (phenolic O-H stretch), 2891.39 (aromatic C-H stretch), 1767.82 (aromatic C=C stretch), 1628.94 (C=O stretch), 1095.60 (C-O stretch), 756.12 (C-I stretch).	9.879(OH, enol, s), 6.899-8.175(2H, Benzene, m), 3.737(3H, Furan, s).	478.1000(M^+), 461.2837, 216.5885, 149.6342, 116.8666($^{1,3}B^+$), 385.2983($^{0,2}A^+$), 200.6396($^{1,3}A^+$), 88.9546($^{0,4}A^+$), 159.7222(M+H-B ring).

6.7.1 Spectral Characterization for Synthesized Compounds

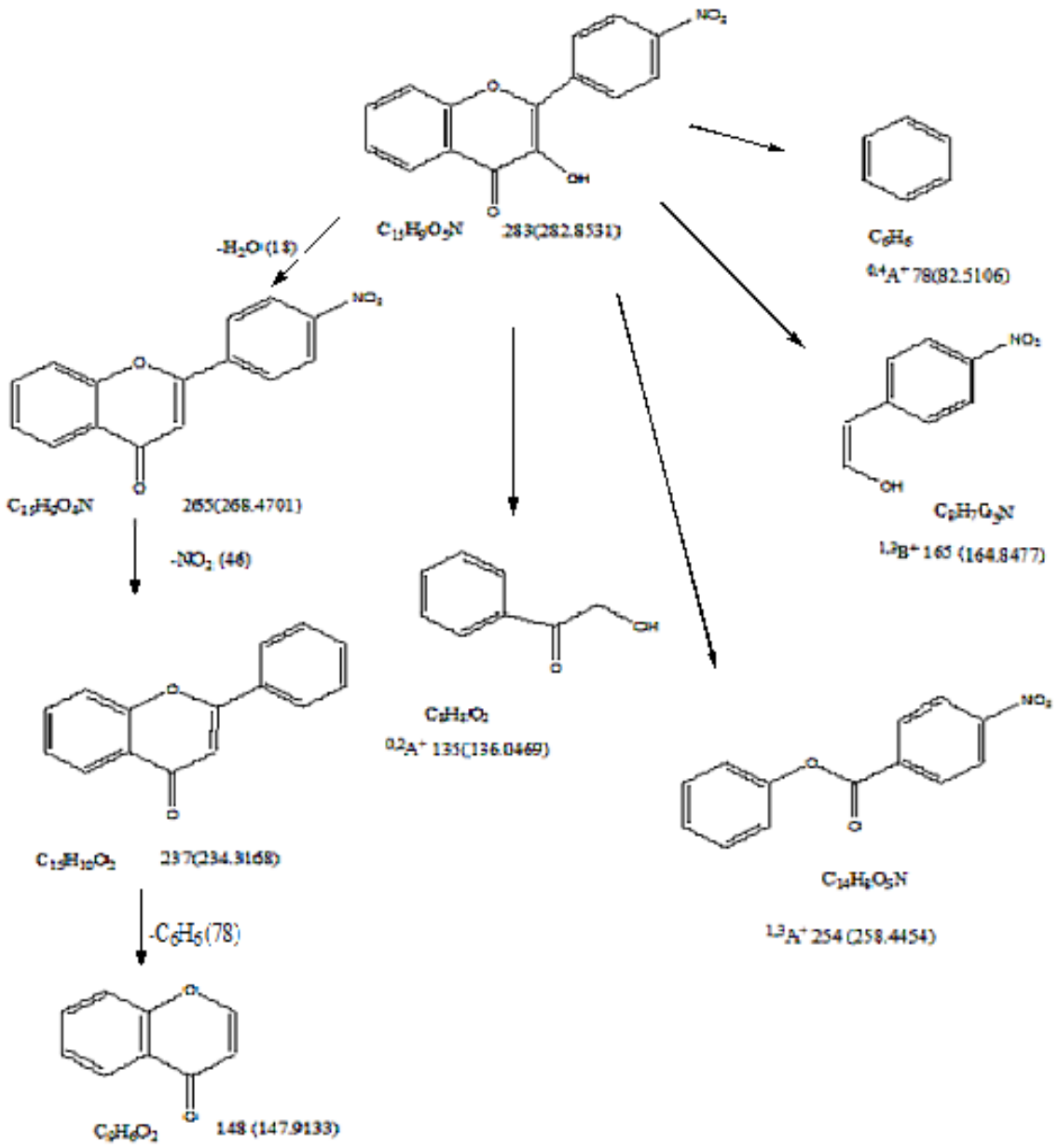
Retrocyclization cleavages of the C-ring

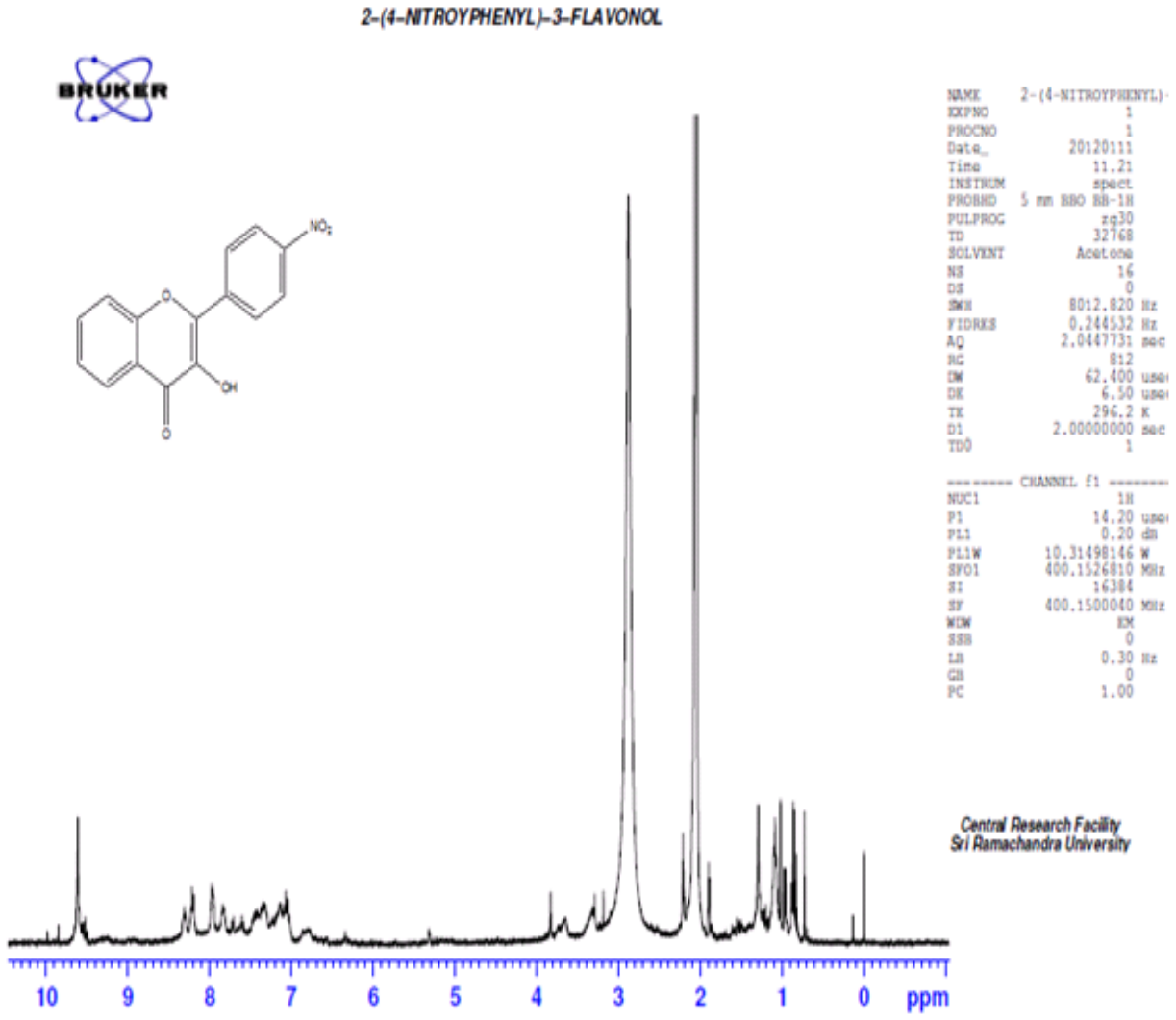


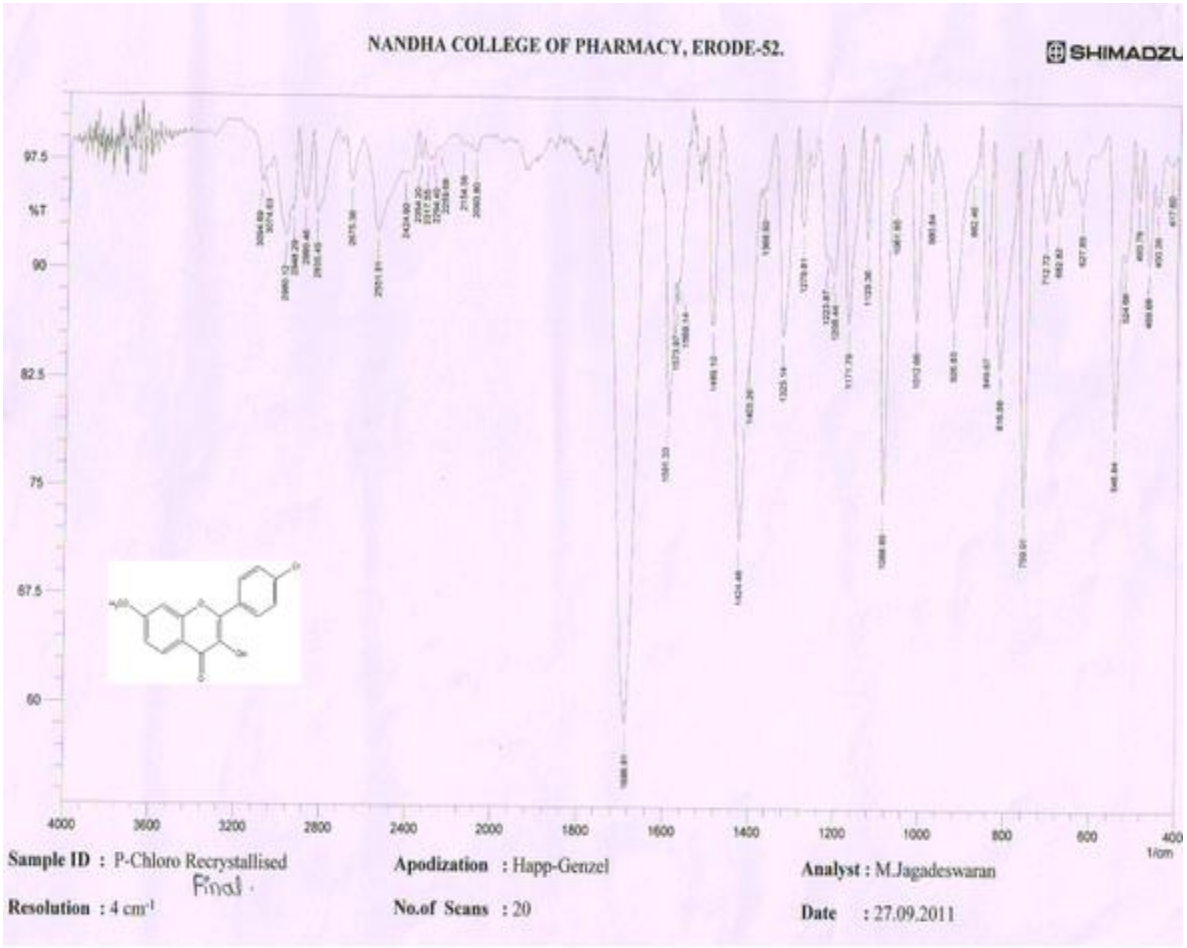

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
105
90
75
60
45
30
3524.06
3429.55
3418.94
3372.65
3358.79
3255.95
3237.63
3077.53
2979.16
2917.43
2899.11
2848.96
1859.44
1809.29
1731.17
1714.77
1696.45
1685.84
1635.69
1600.97
1556.61
1521.89
1488.13
1475.59
1464.02
1415.80
1395.54
1349.25
1312.60
1262.45
1237.38
1213.27
1178.55
1165.04
1157.33
1111.03
1085.96
1031.95
1014.59
988.52
984.69
910.43
892.11
854.49
836.17
780.23
754.19
617.24
515.98
470.65
458.07
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
1/cm
Sample ID : nitro final
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 30.08.2011

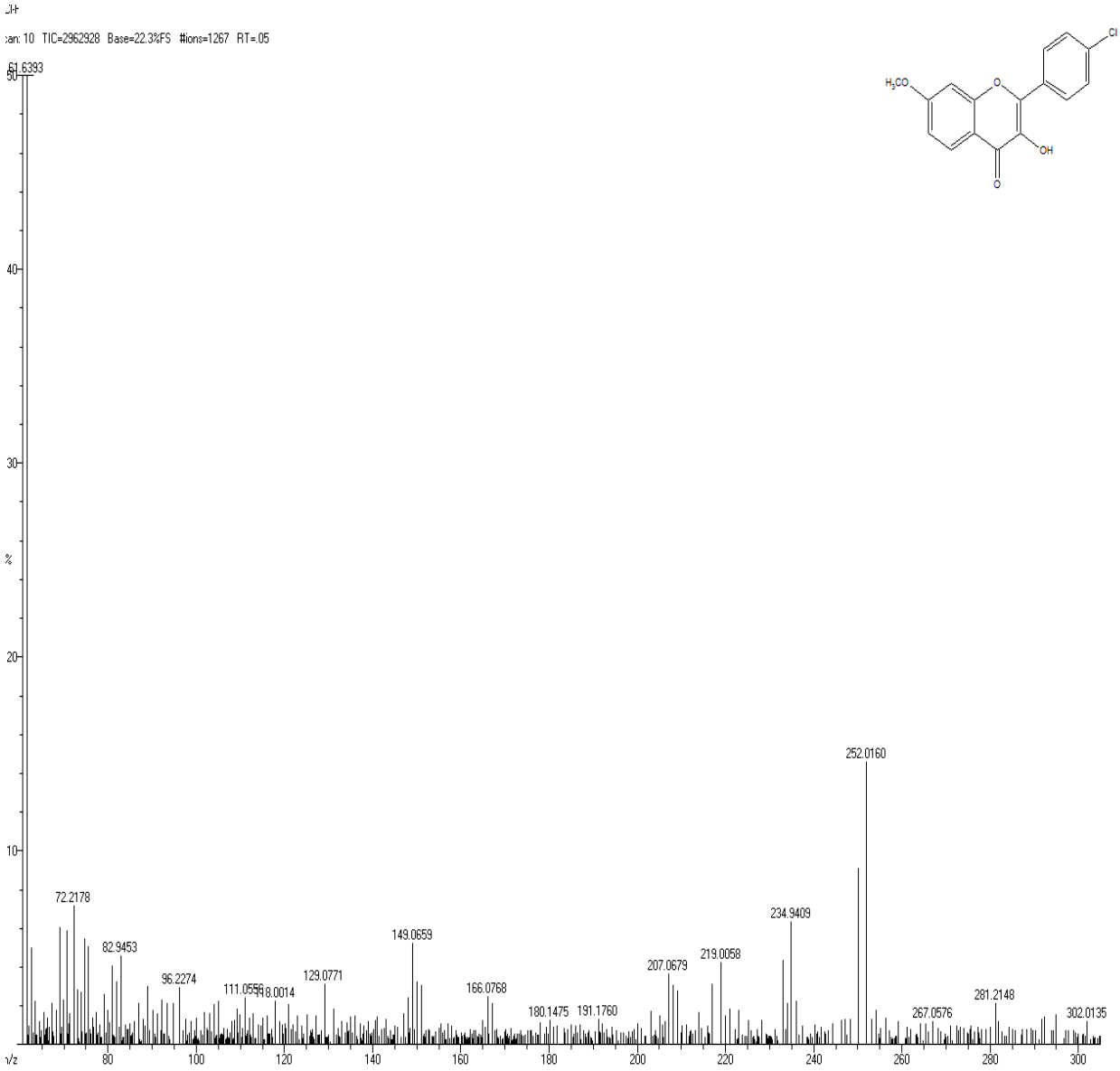

N-F
Scan: 8 TIC=4504576 Base=20.5%FS #Ions=1727 RT=.04
58.8943
72.5684
82.5106
90.4009
104.2924
110.2654
128.1173
136.0469
147.9133
154.8973
164.8477
183.7624
194.5042
203.4222
211.6751
226.4588
234.3168
250.0387
258.4454
268.4701
282.8531
%
m/z
NO2
OH
O

COMPOUND A:

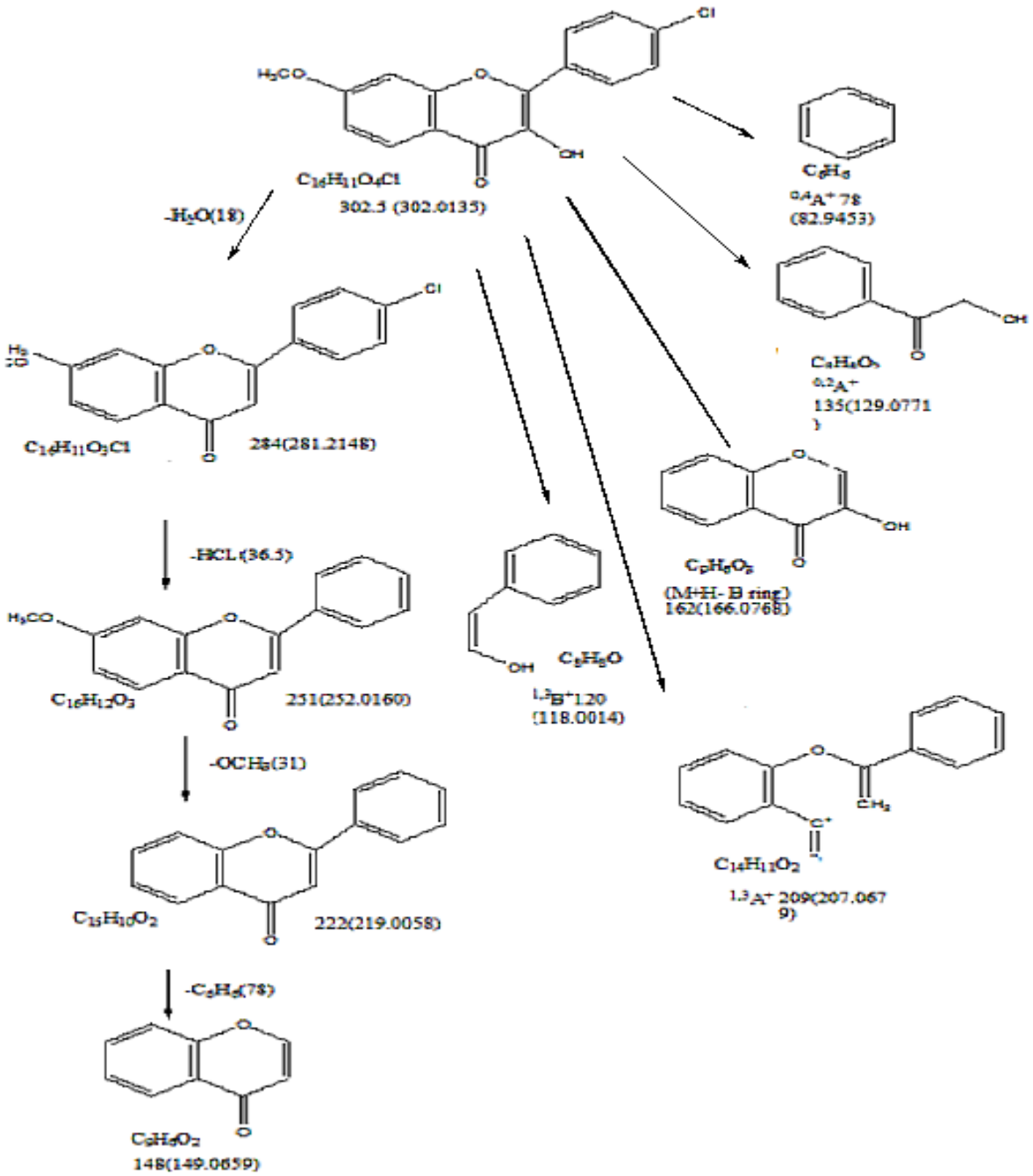

$C_{15}H_9O_5N$ 283(282.8531)
$-H_2O$ (18)
$C_{15}H_7O_4N$ 265(268.4701)
$-NO_2$ (46)
$C_{15}H_{10}O_2$ 237(234.8168)
$-C_6H_5$(78)
$C_9H_6O_2$ 148 (147.9133)
C_6H_5
$^{0,4}A^+$ 78(82.5106)
$C_9H_7O_3N$
$^{1,3}B^+$ 165 (164.8477)
$C_9H_5O_2$
$^{0,2}A^+$ 135(136.0469)
$C_{14}H_8O_5N$
$^{1,3}A^+$ 254 (258.4454)

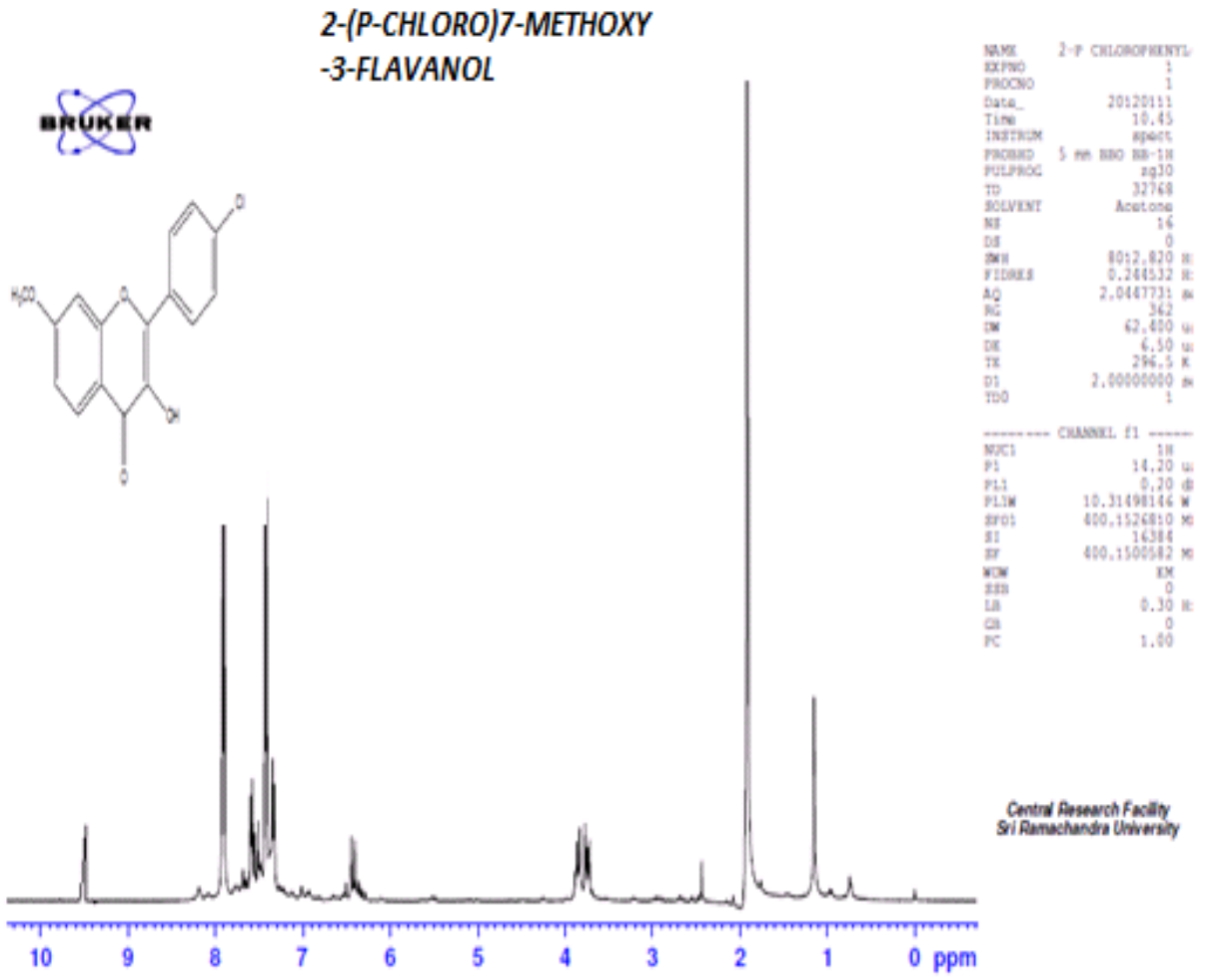

2-(4-NITROYPHENYL)-3-FLAVONOL
BRUKER
NAME 2-(4-NITROYPHENYL)-
EXPNO 1
PROCNO 1
Date_ 20120111
Time 11.21
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 812
DW 62.400 usec
DE 6.50 usec
TE 296.2 K
D1 2.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 14.20 usec
PL1 0.20 dB
PL1W 10.31498146 W
SFO1 400.1526810 MHz
SI 16384
SF 400.1500040 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
10 9 8 7 6 5 4 3 2 1 0 ppm

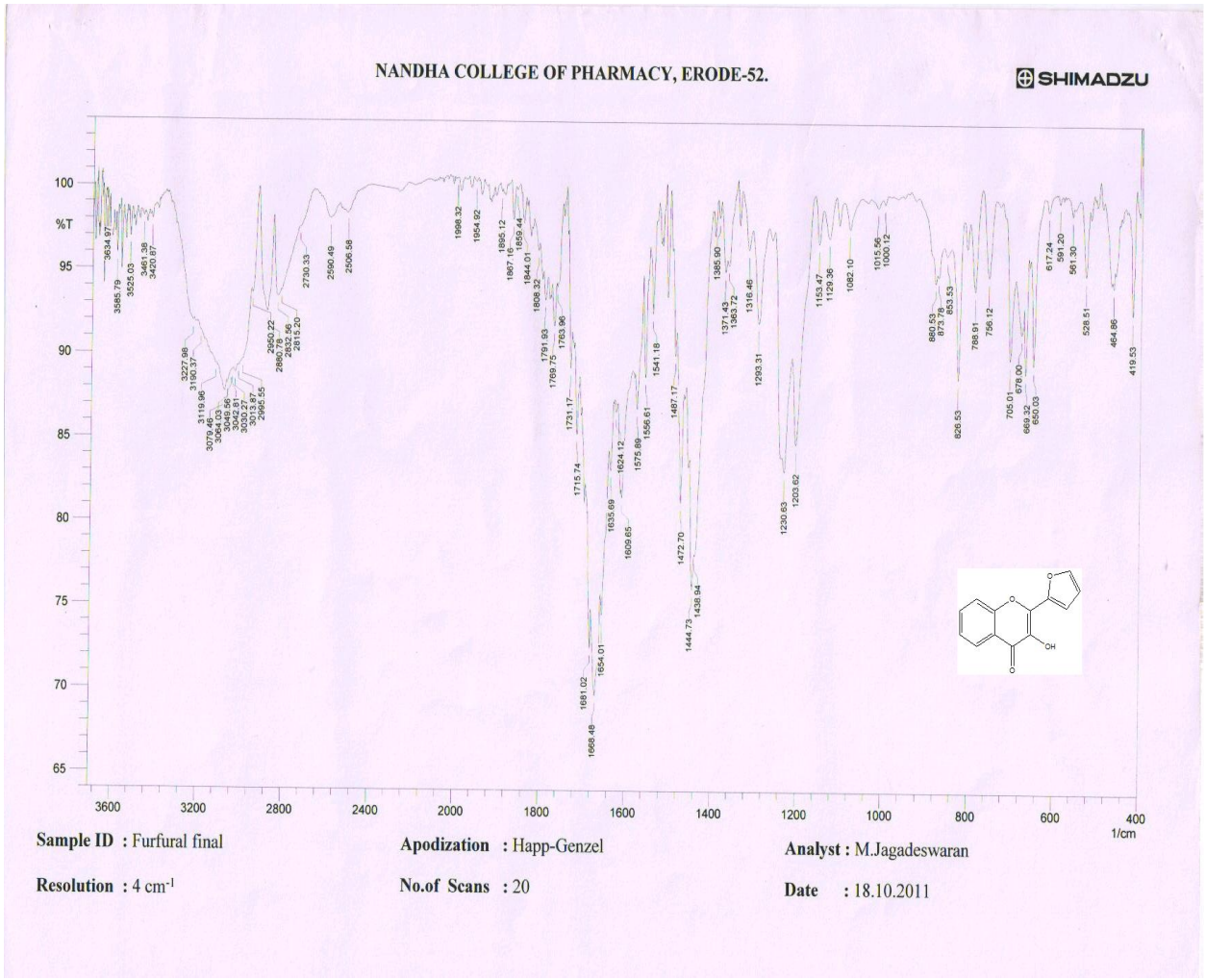

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
97.5
%T
90
82.5
75
67.5
60
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
Sample ID : P-Chloro Recrystallised
Final
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 27.09.2011

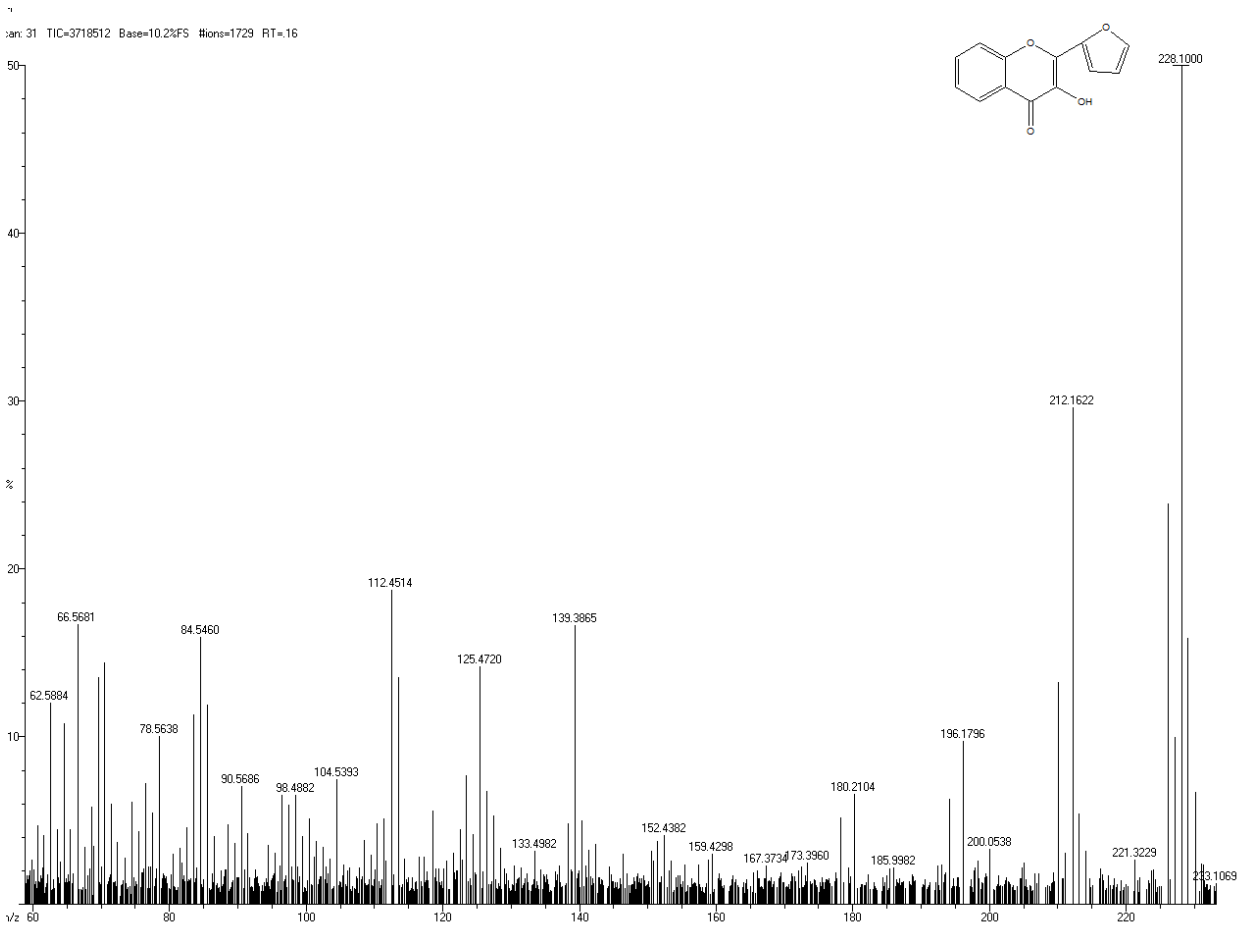

Cl
H3CO
O
OH
O
252.0160
72.2178
82.9453
96.2274
111.0556
118.0014
129.0771
149.0659
166.0768
180.1475
191.1760
207.0679
219.0058
234.9409
267.0576
281.2148
302.0135
%
m/z

COMPOUND B

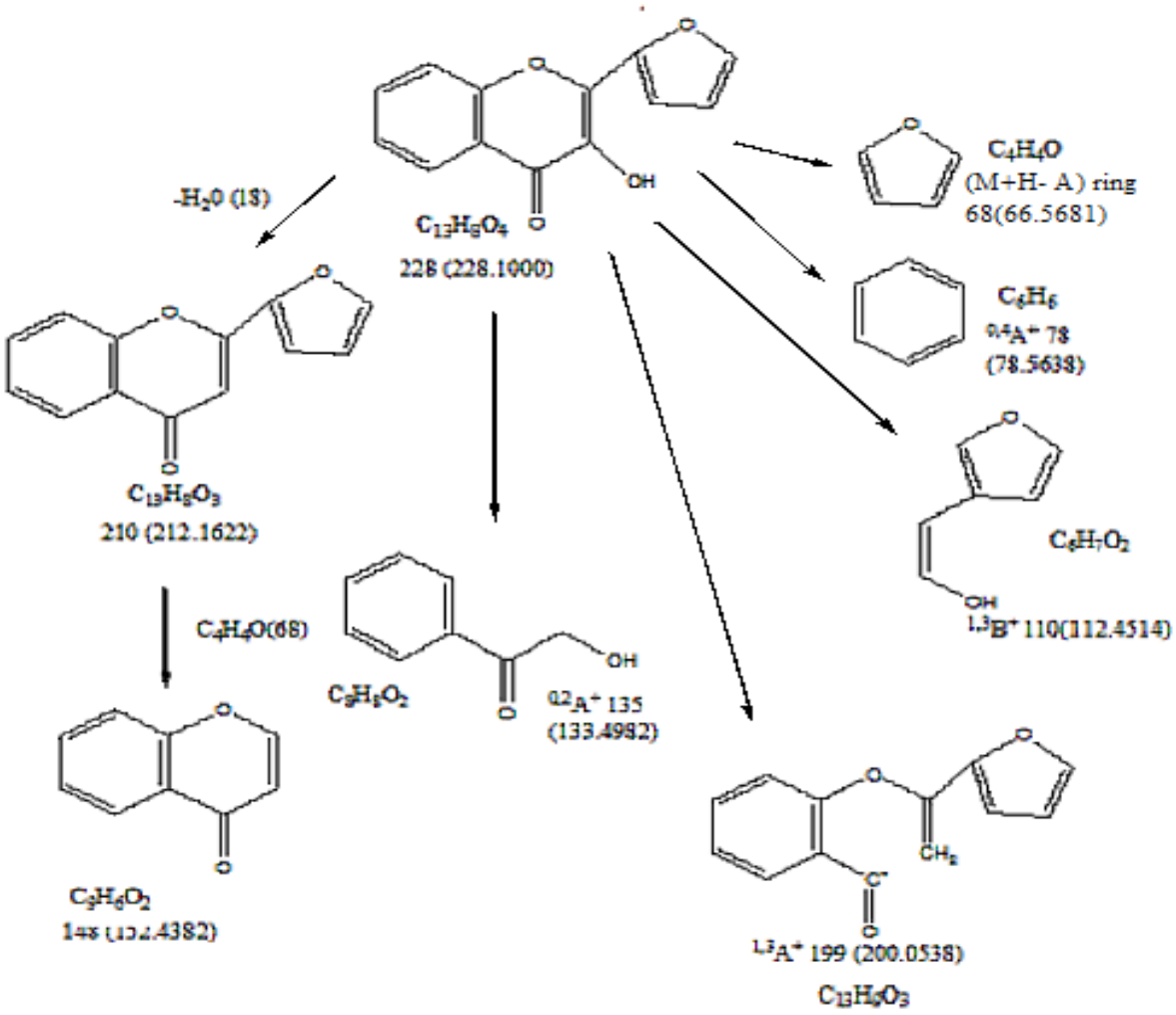

Cl
H3CO
O
OH
C16H11O4Cl
302.5 (302.0135)
-H2O(18)
C6H6
0.4A+ 78
(82.9453)
OH
C8H8O2
0.2A+
135(129.0771
)
Cl
H3
CO
O
C16H11O3Cl
284(281.2148)
OH
C9H8O2
(M+H- B ring)
162(166.0768)
-HCL(36.5)
H3CO
O
C16H12O3
251(252.0160)
OH
C8H8O
1.3B+120
(118.0014)
-OCH3(31)
CH2
C+
O
C14H11O2
1.3A+ 209(207.067
9)
O
C15H10O2
222(219.0058)
-C6H6(78)
O
C9H6O2
148(149.0659)

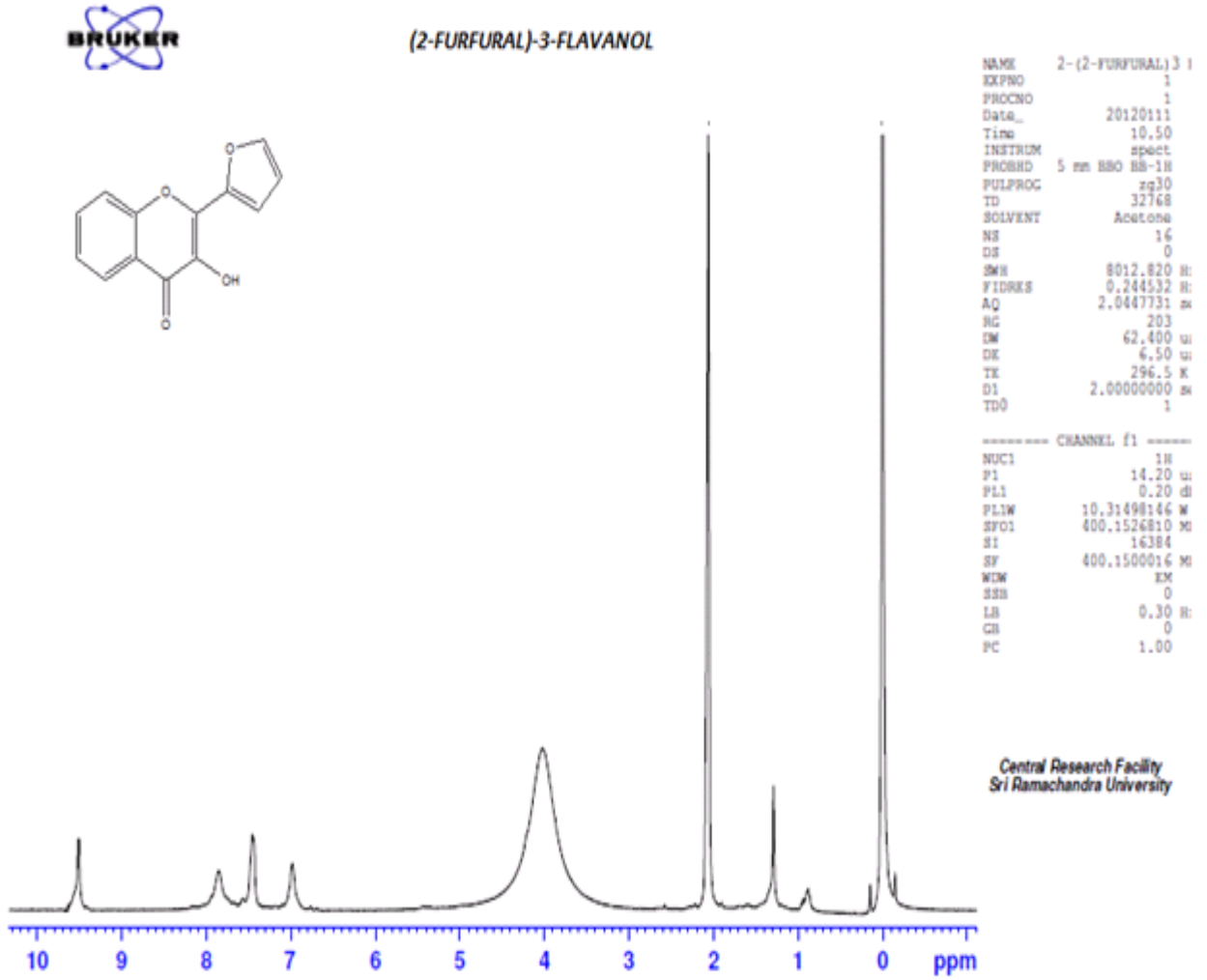

2-(P-CHLORO)7-METHOXY
-3-FLAVANOL
BRUKER
NAME 2-P CHLOROPHENYL
EXPNO 1
PROCNO 1
Date_ 20120111
Time 10.45
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 362
DW 62.400 usec
DE 6.50 usec
TE 296.5 K
D1 2.00000000 sec
TD0 1
---------- CHANNEL f1 ------
NUC1 1H
P1 14.20 usec
PL1 0.20 dB
PL1W 10.31498146 W
SFO1 400.1526810 MHz
SI 16384
SF 400.1500582 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
10 9 8 7 6 5 4 3 2 1 0 ppm

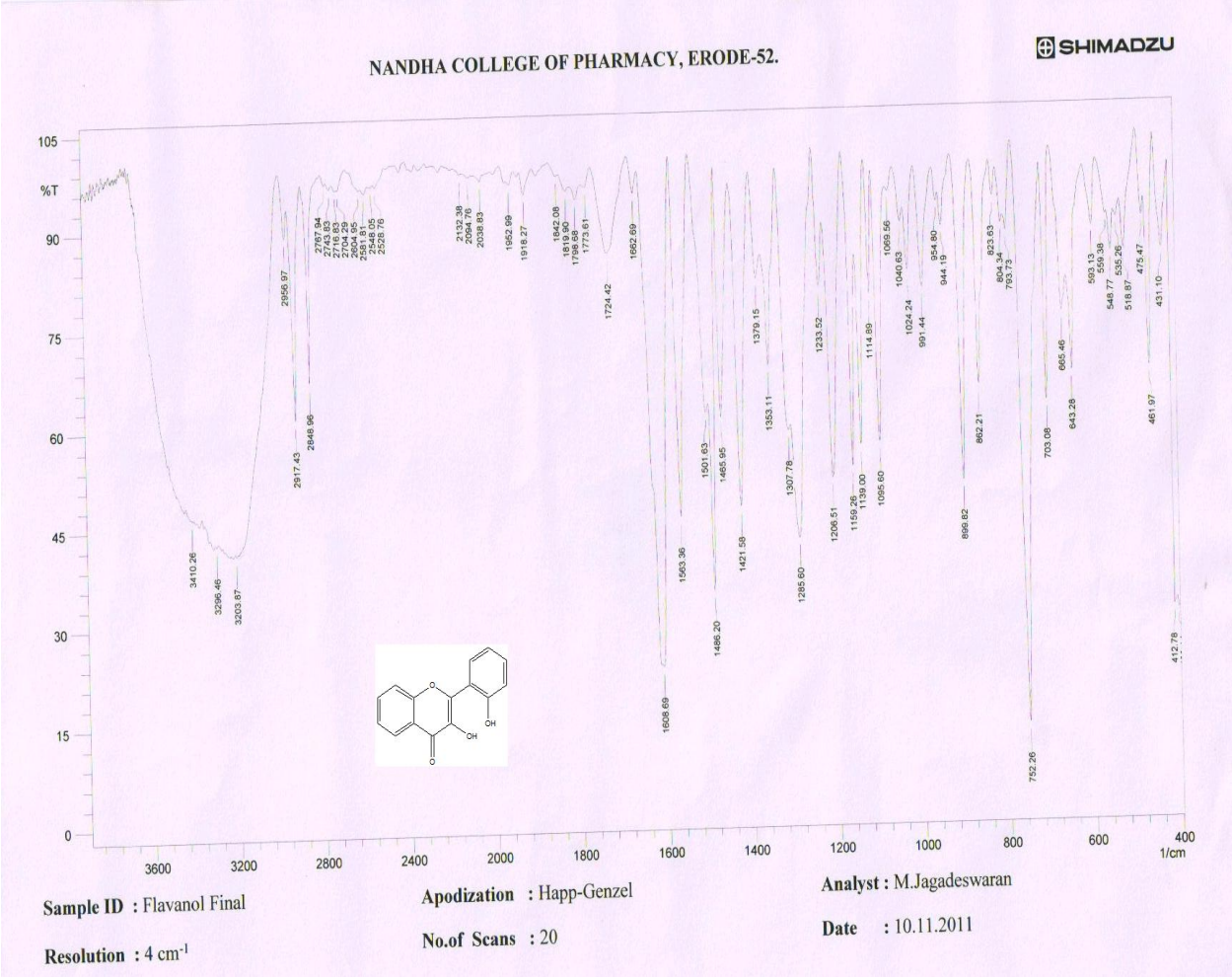

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
1/cm
Sample ID : Furfural final
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 18.10.2011

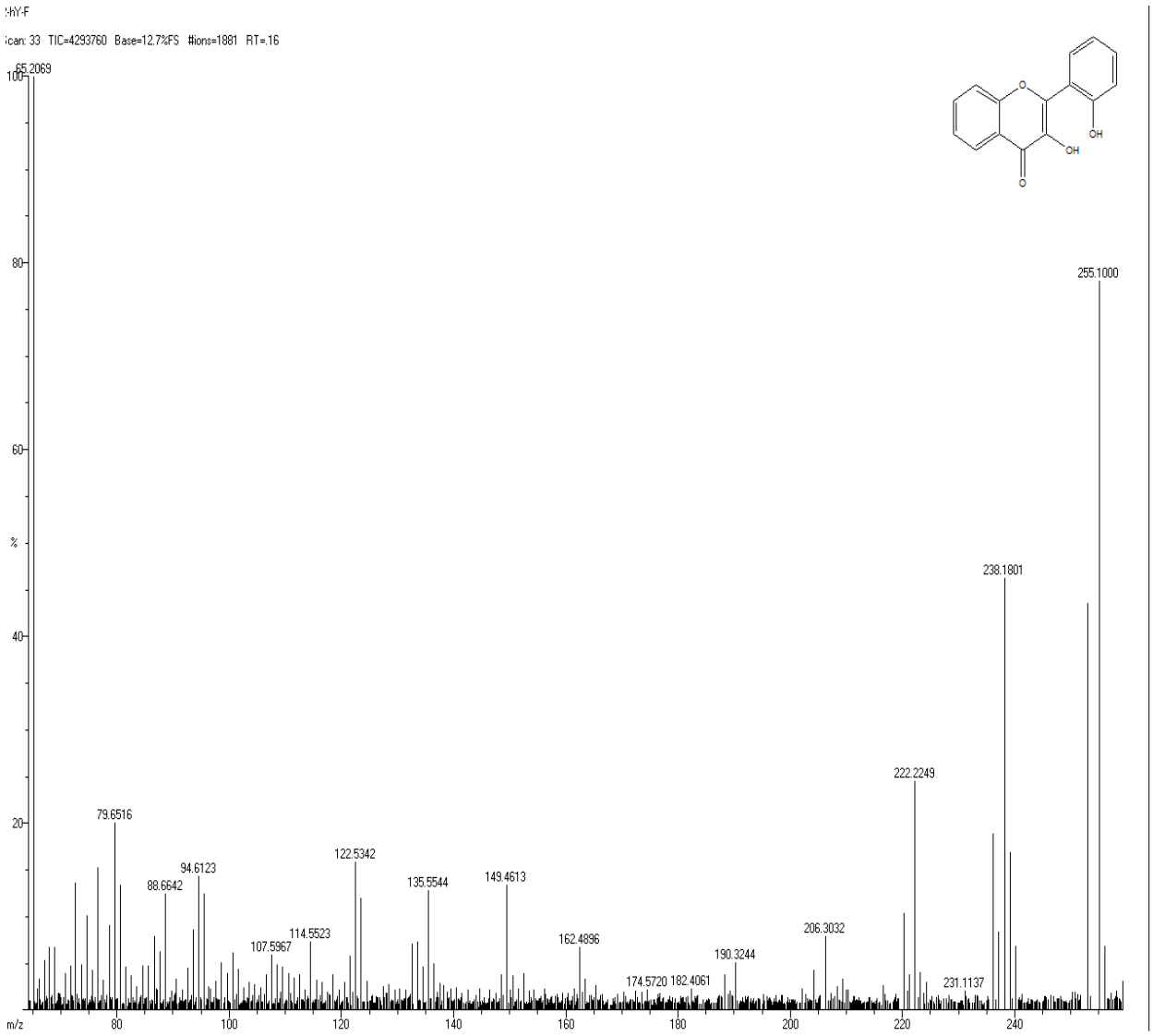

Scan: 31 TIC=3718512 Base=10.2%FS #ions=1729 RT=.16
228.1000
212.1622
112.4514
66.5681
84.5460
139.3865
125.4720
62.5884
78.5638
196.1796
90.5686
98.4882
104.5393
180.2104
152.4382
133.4982
159.4298
167.3734
173.3960
200.0538
185.9982
221.3229
223.1069
%
m/z
OH

COMPOUND C

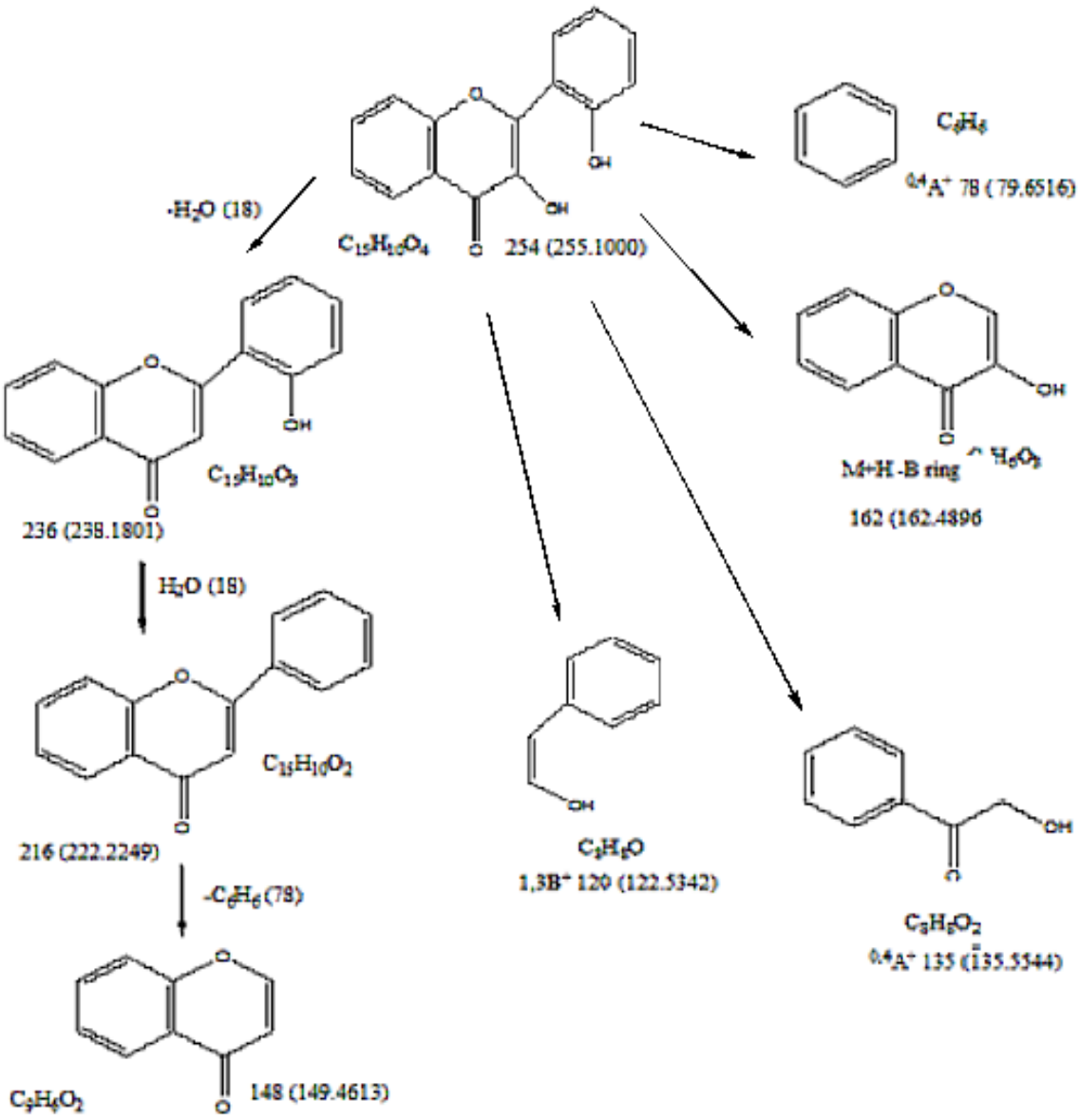

-H2O (18)
C13H8O4
228 (228.1000)
OH
C4H4O
(M+H- A) ring
68(66.5681)
C6H6
0,4A+ 78
(78.5638)
C13H8O3
210 (212.1622)
C6H7O2
OH
1,3B+ 110(112.4514)
C4H4O(68)
C9H5O2
OH
0,2A+ 135
(133.4982)
CH2
C+
1,3A+ 199 (200.0538)
C13H9O3

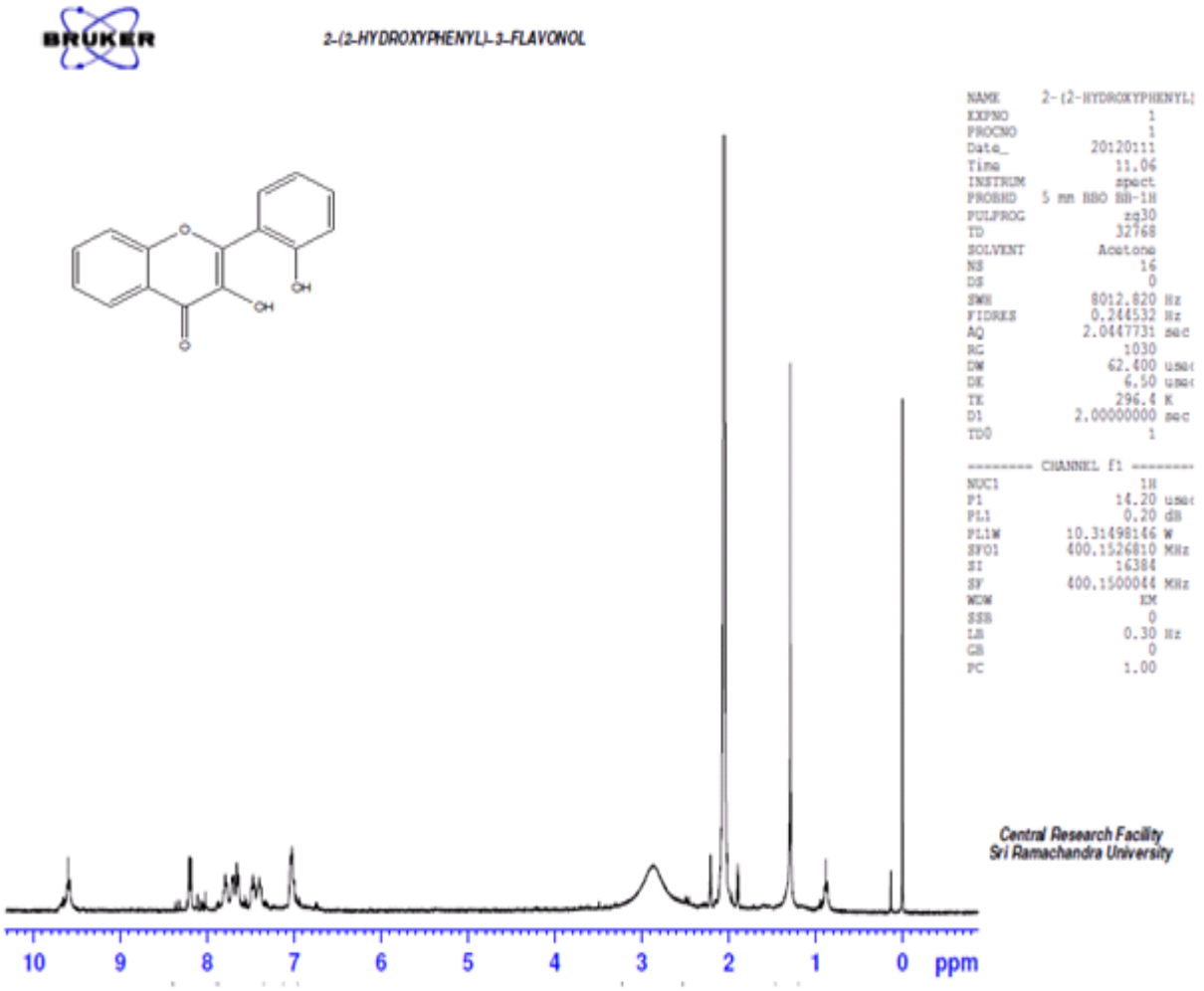

BRUKER
(2-FURFURAL)-3-FLAVANOL
OH
O
O
O
NAME 2-(2-FURFURAL)3 1
EXPNO 1
PROCNO 1
Date_ 20120111
Time 10.50
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 8012.820 H
FIDRES 0.244532 H
AQ 2.0447731 s
RG 203
DW 62.400 u
DE 6.50 u
TE 296.5 K
D1 2.00000000 s
TD0 1
CHANNEL f1
NUC1 1H
P1 14.20 u
PL1 0.20 d
PL1W 10.31498146 W
SFO1 400.1526810 M
SI 16384
SF 400.1500016 M
WDW EM
SSB 0
LB 0.30 H
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
10
9
8
7
6
5
4
3
2
1
0
ppm

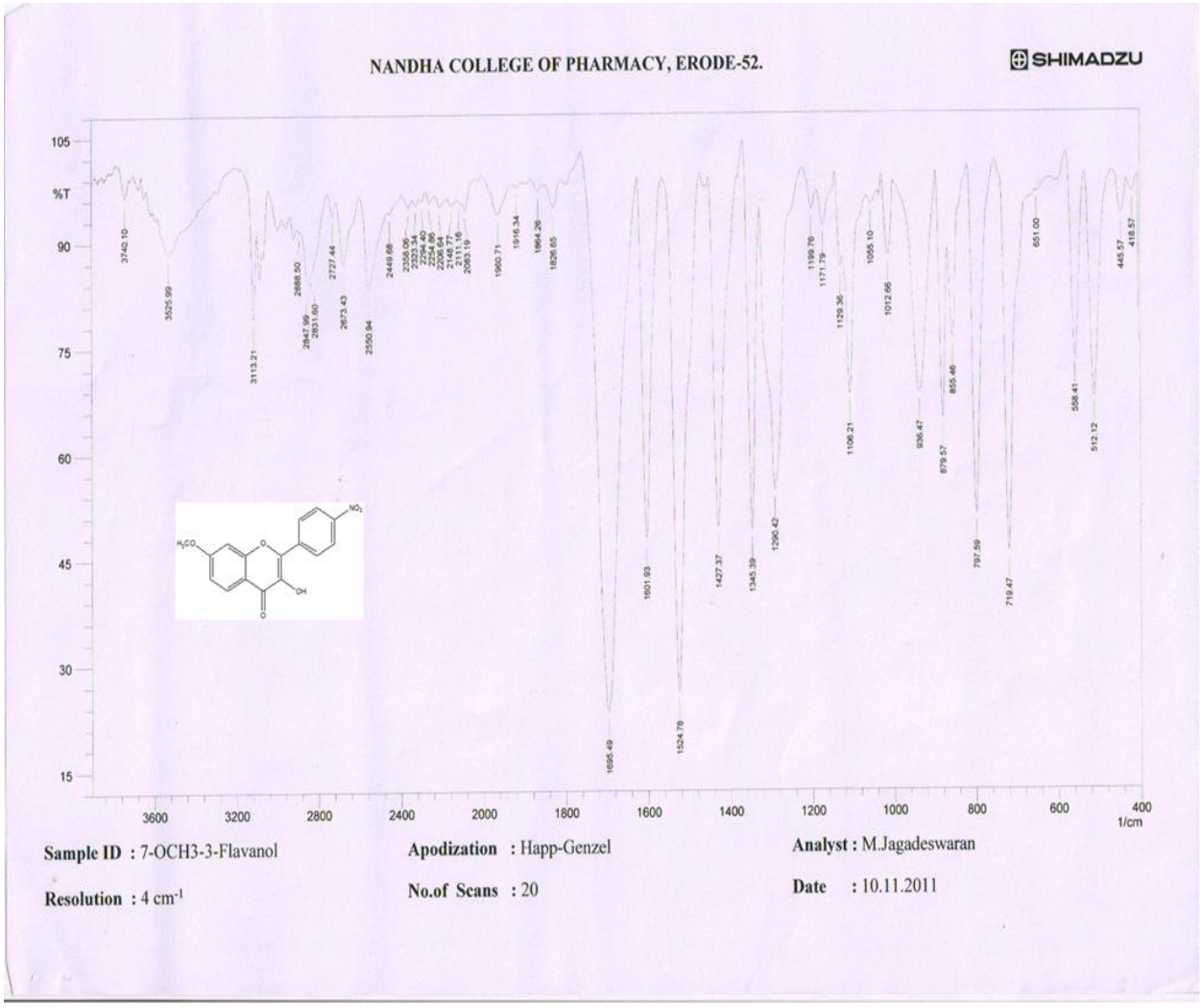

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
1/cm
Sample ID : Flavanol Final
Resolution : 4 cm-1
Apodization : Happ-Genzel
No.of Scans : 20
Analyst : M.Jagadeswaran
Date : 10.11.2011

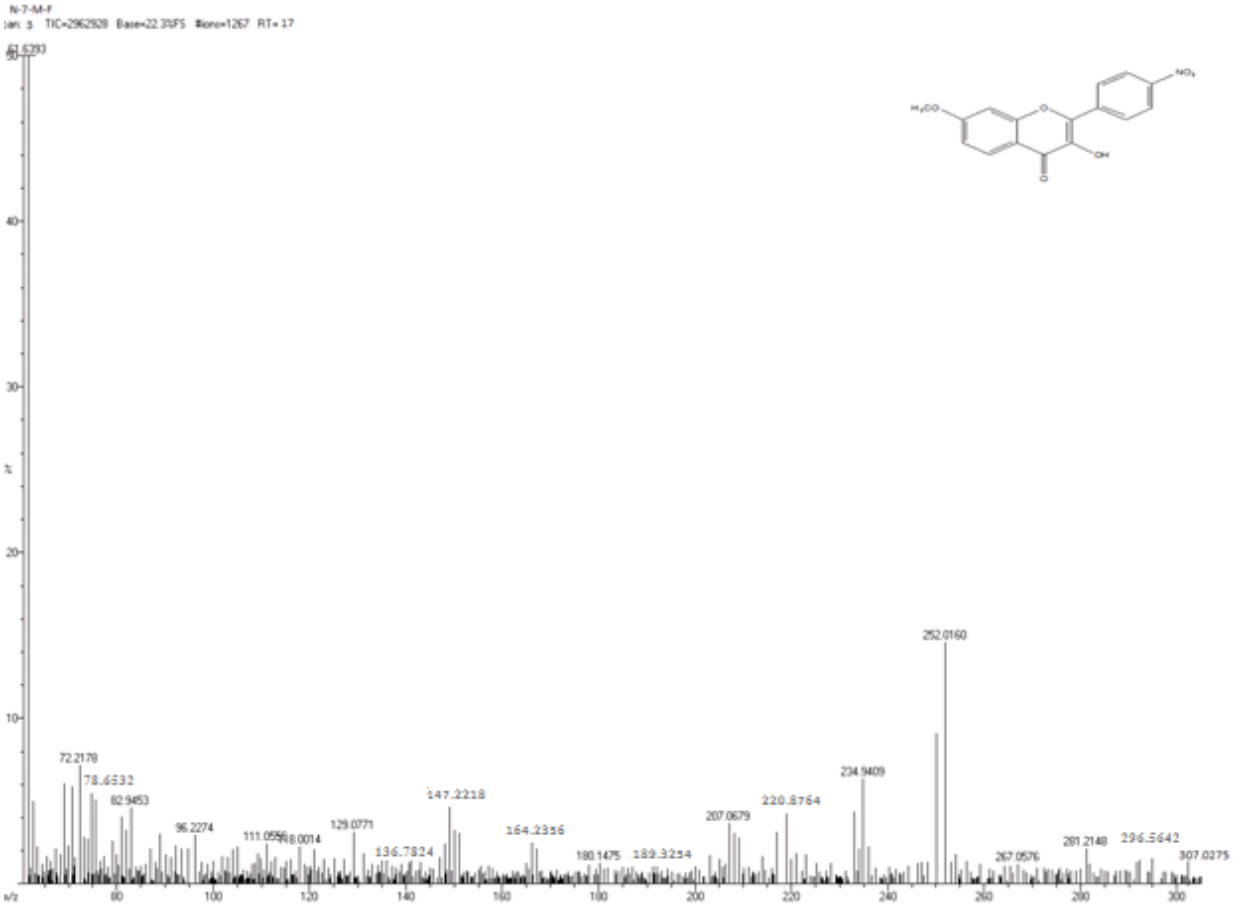

:hY-F
ican: 33 TIC=4293760 Base=12.7%FS #ions=1881 RT=.16
65.2069
255.1000
238.1801
222.2249
79.6516
94.6123
88.6642
122.5342
135.5544
149.4613
114.5523
107.5967
162.4896
206.3032
190.3244
174.5720
182.4061
231.1137
%
m/z
OH
OH
O
O

COMPOUND D

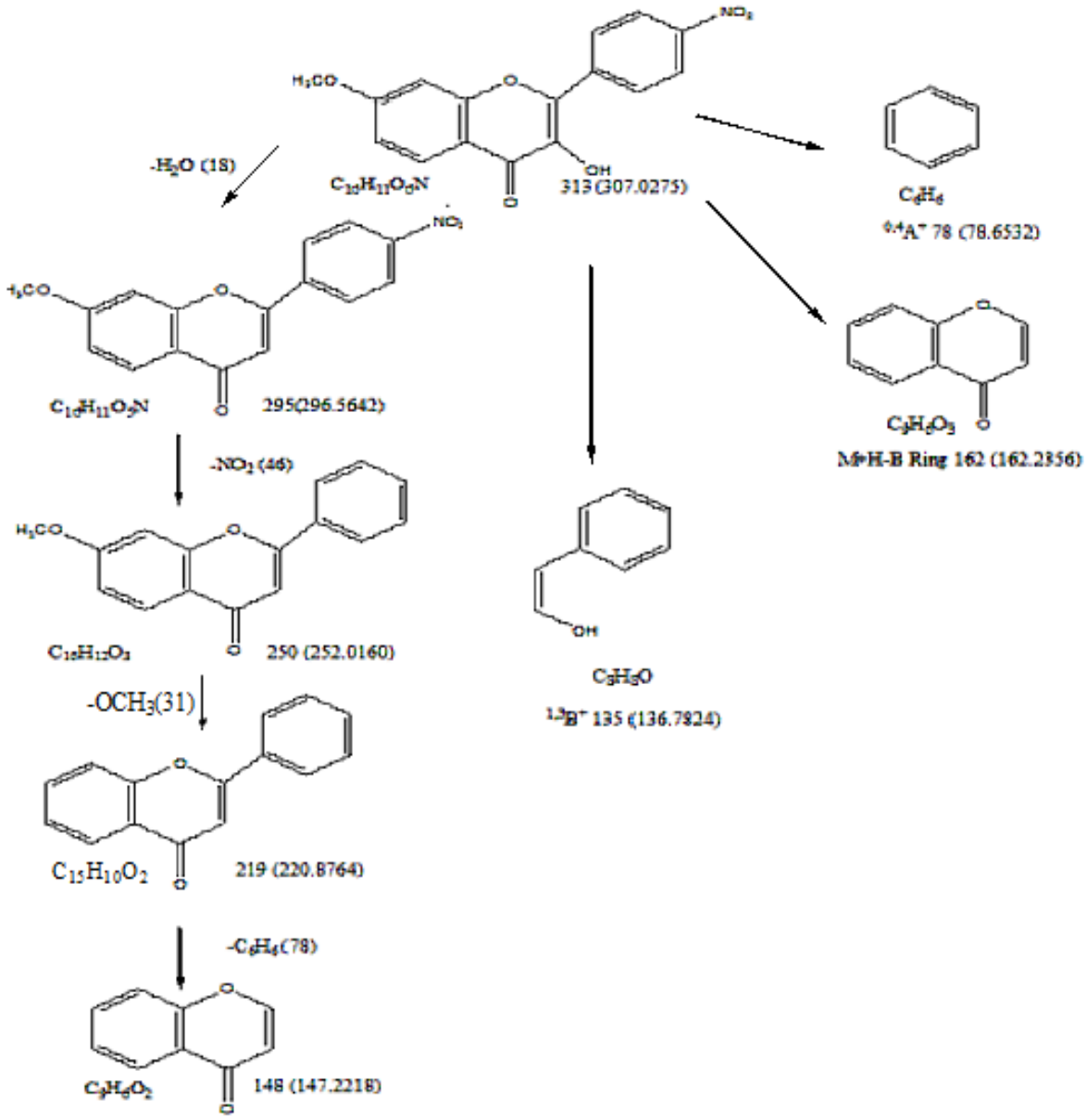

-H2O (18)
C15H16O4
254 (255.1000)
C6H6
0,4A+ 78 (79.6516)
C15H10O3
236 (238.1801)
M+H -B ring
^ H6O2
162 (162.4896
H2O (18)
C15H10O2
216 (222.2249)
-C6H6 (78)
C9H6O2
148 (149.4613)
C3H5O
1,3B+ 120 (122.5342)
C8H5O2
0,4A+ 135 (135.5544)

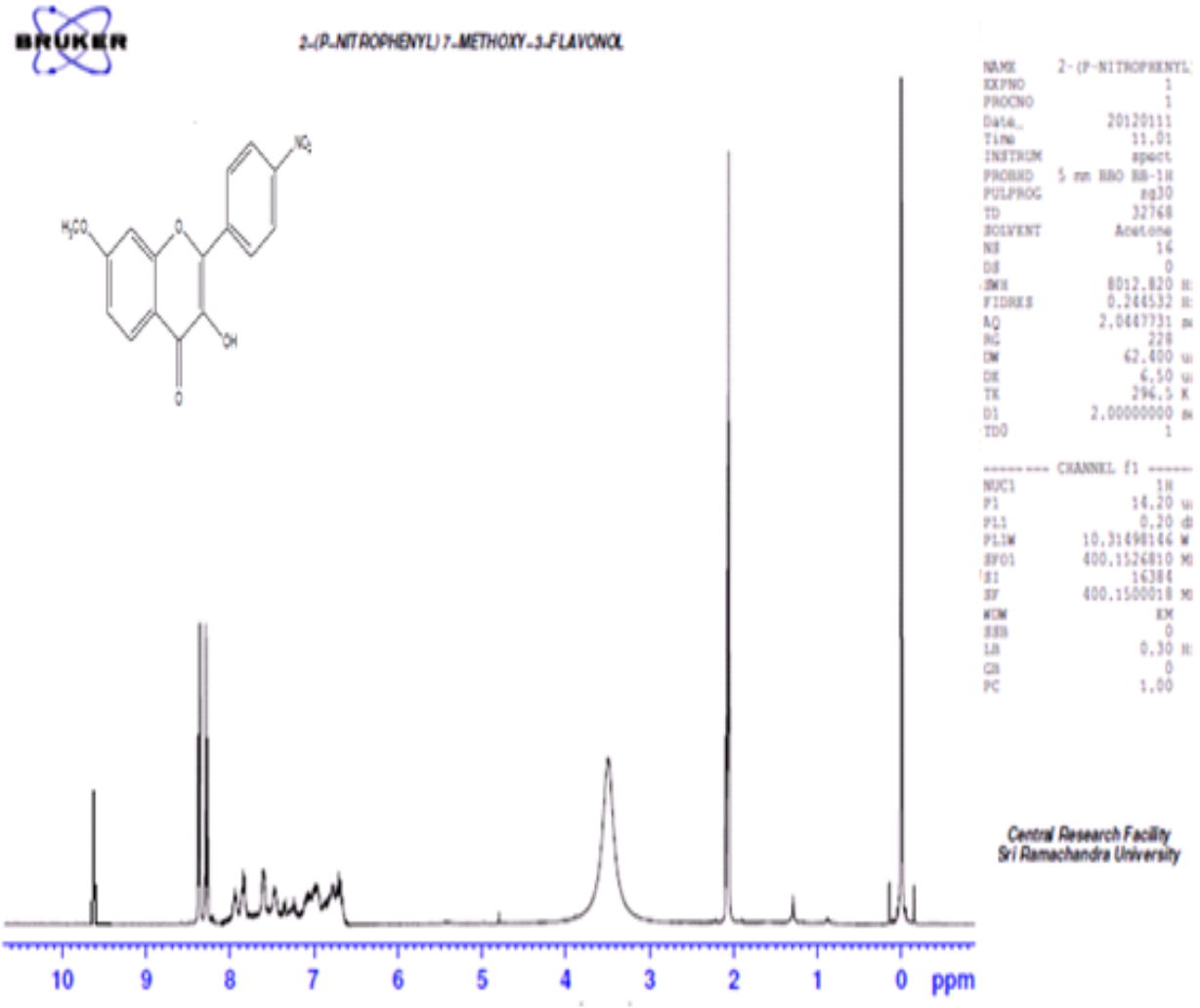

BRUKER
2-(2-HYDROXYPHENYL)-3-FLAVONOL
OH
OH
NAME 2-(2-HYDROXYPHENYL)
EXPNO 1
PROCNO 1
Date_ 20120111
Time 11.06
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 1030
DW 62.400 use
DE 6.50 use
TE 296.4 K
D1 2.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 14.20 use
PL1 0.20 dB
PL1W 10.31498146 W
SFO1 400.1526810 MHz
SI 16384
SF 400.1500044 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
10 9 8 7 6 5 4 3 2 1 0 ppm

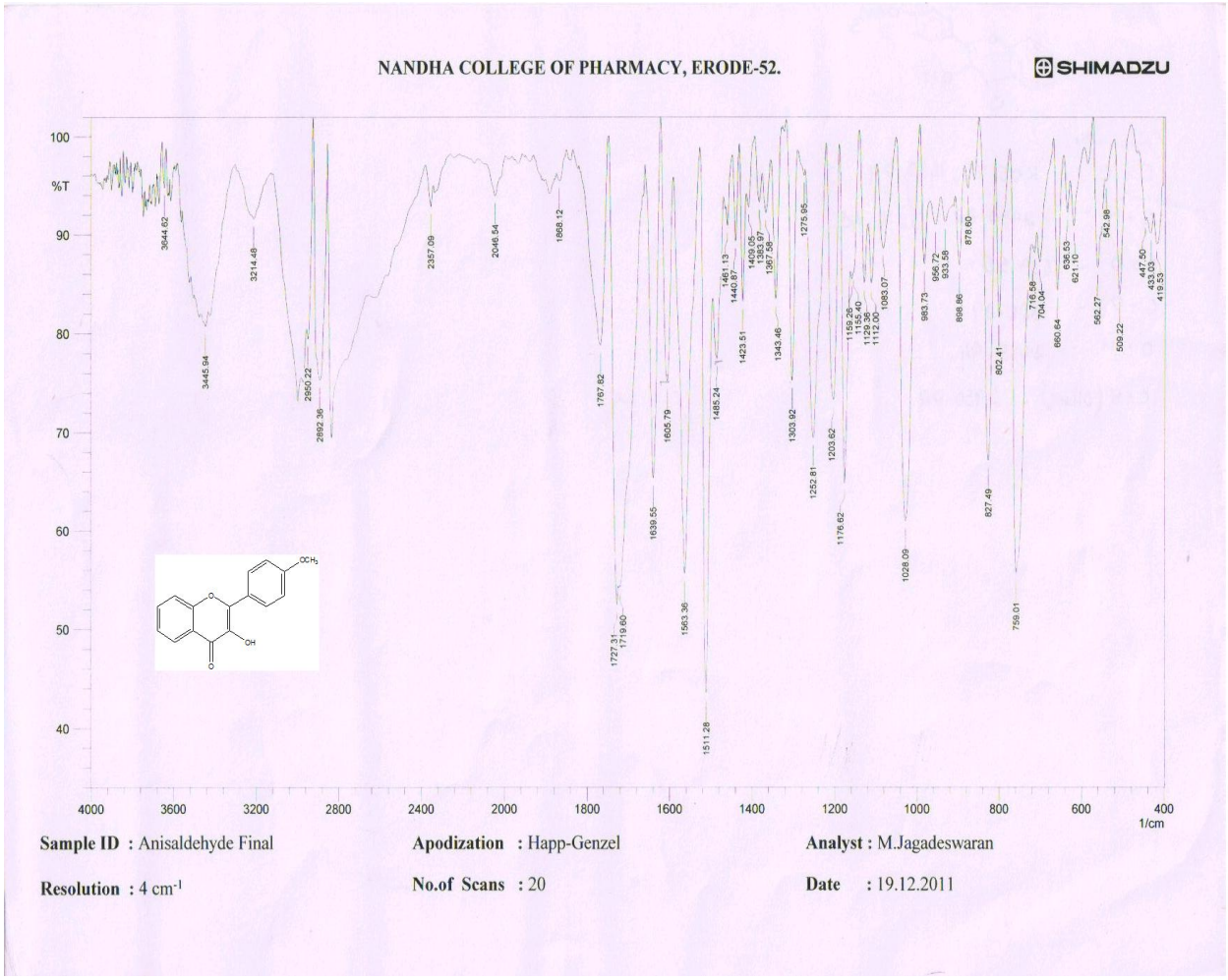

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
1/cm
Sample ID : 7-OCH3-3-Flavanol
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 10.11.2011

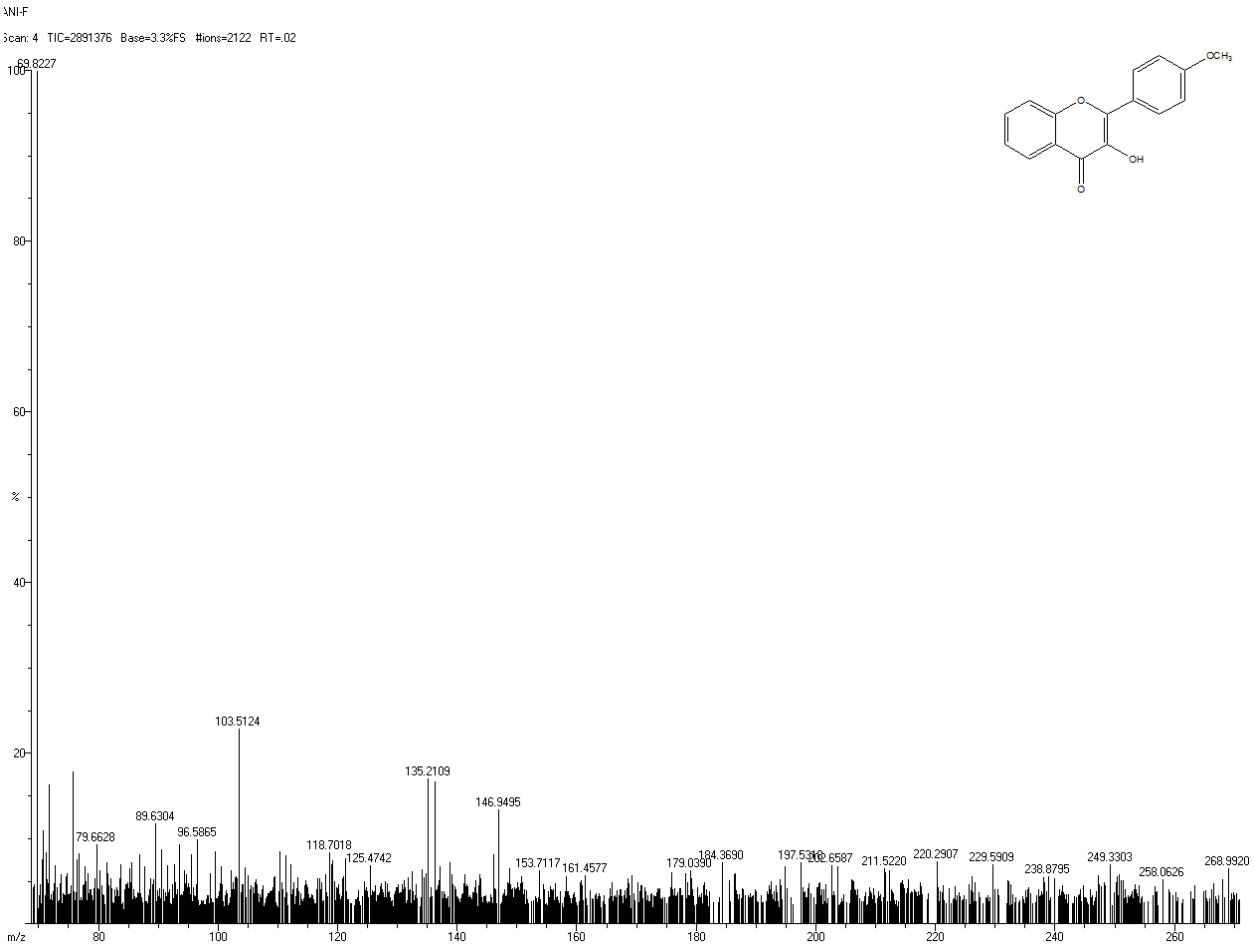

N-7-M-F
TIC=2962928 Base=22.3%FS #Ions=1267 RT=17
41.6293
72.2178
78.6532
82.9453
96.2274
111.0556
118.0014
129.0771
136.7824
147.2218
164.2356
180.1475
189.3254
207.0679
220.8764
234.9409
252.0160
267.0576
281.2148
296.5642
307.0275
%
m/z

COMPOUND E

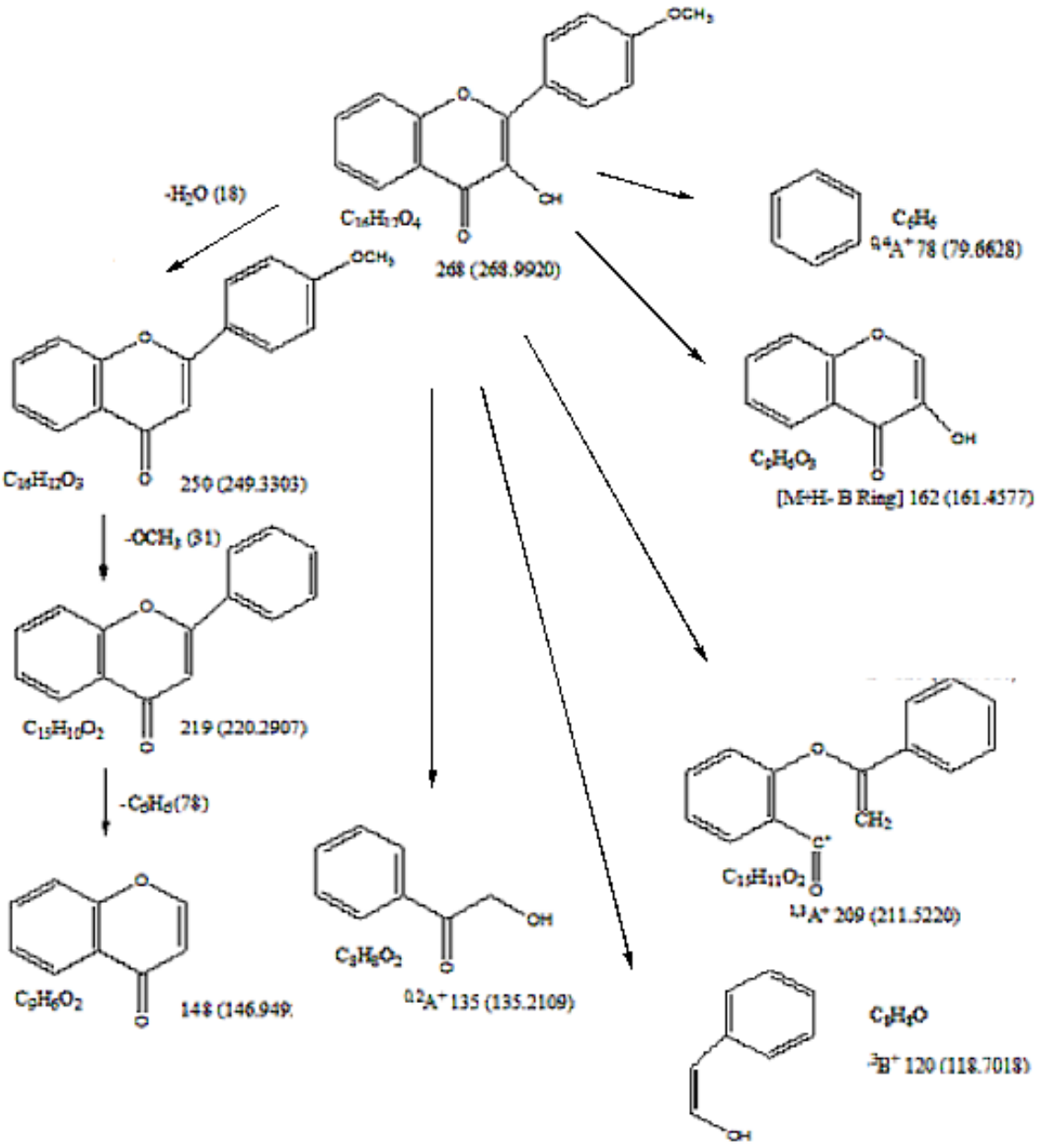

-H2O (18)
C15H11O5N
313 (307.0275)
C6H6
A+ 78 (78.6532)
C15H11O5N
295(296.5642)
C3H5O3
M+H-B Ring 162 (162.2356)
-NO2 (46)
C15H12O3
250 (252.0160)
C3H5O
B+ 135 (136.7824)
-OCH3(31)
C15H10O2
219 (220.8764)
-C6H4(78)
C9H6O2
148 (147.2218)

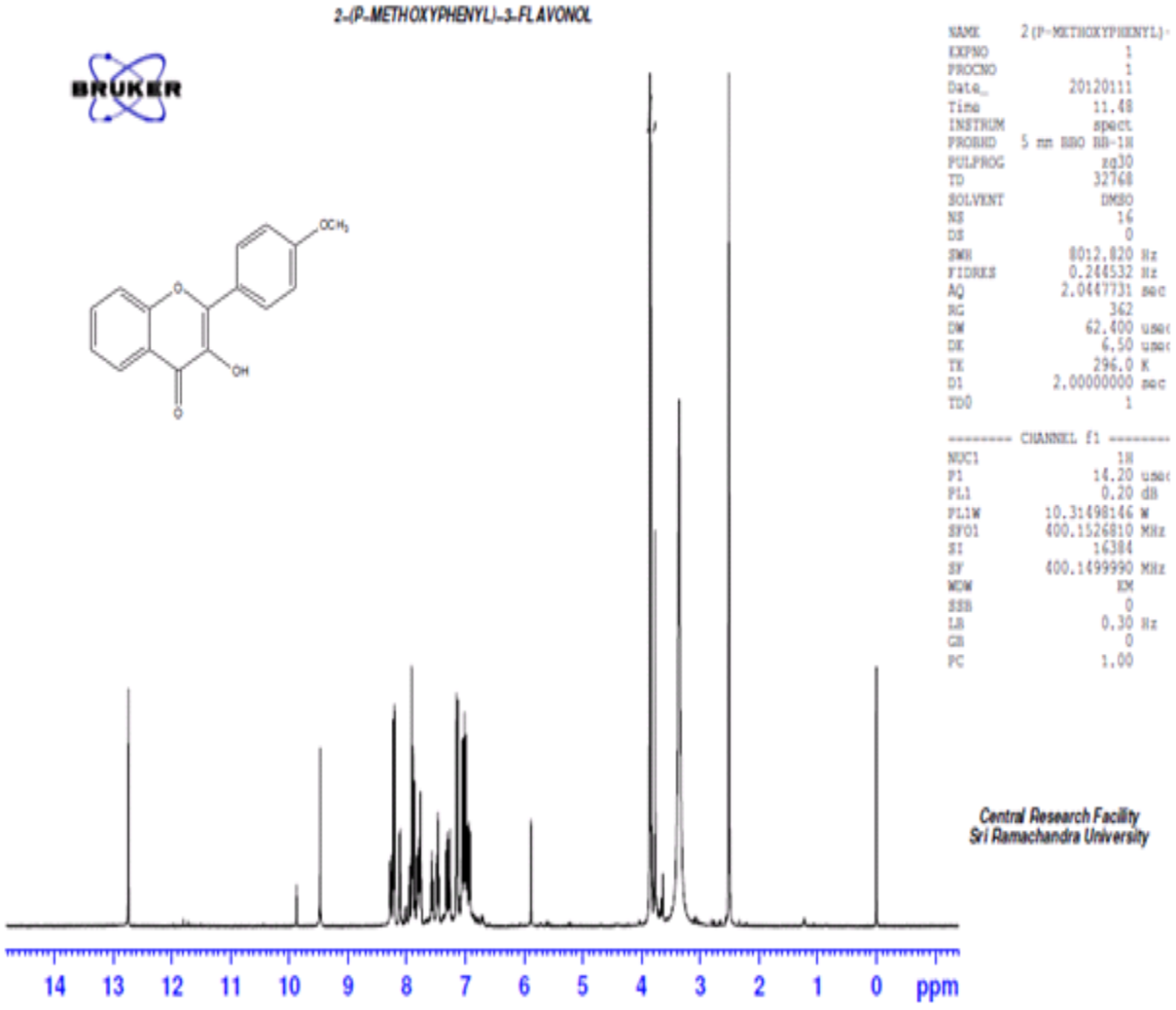

BRUKER
2-(P-NITROPHENYL) 7-METHOXY-3-FLAVONOL
NAME 2-(P-NITROPHENYL
EXPNO 1
PROCNO 1
Date_ 20120111
Time 11.01
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 8012.820 H
FIDRES 0.244532 H
AQ 2.0447731 s
RG 228
DW 62.400 u
DE 6.50 u
TE 296.5 K
D1 2.00000000 s
TD0 1
CHANNEL f1
NUC1 1H
P1 14.20 u
PL1 0.20 d
PL1W 10.31498146 W
SFO1 400.1526810 M
SI 16384
SF 400.1500018 M
WDW EM
SSB 0
LB 0.30 H
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
10 9 8 7 6 5 4 3 2 1 0 ppm

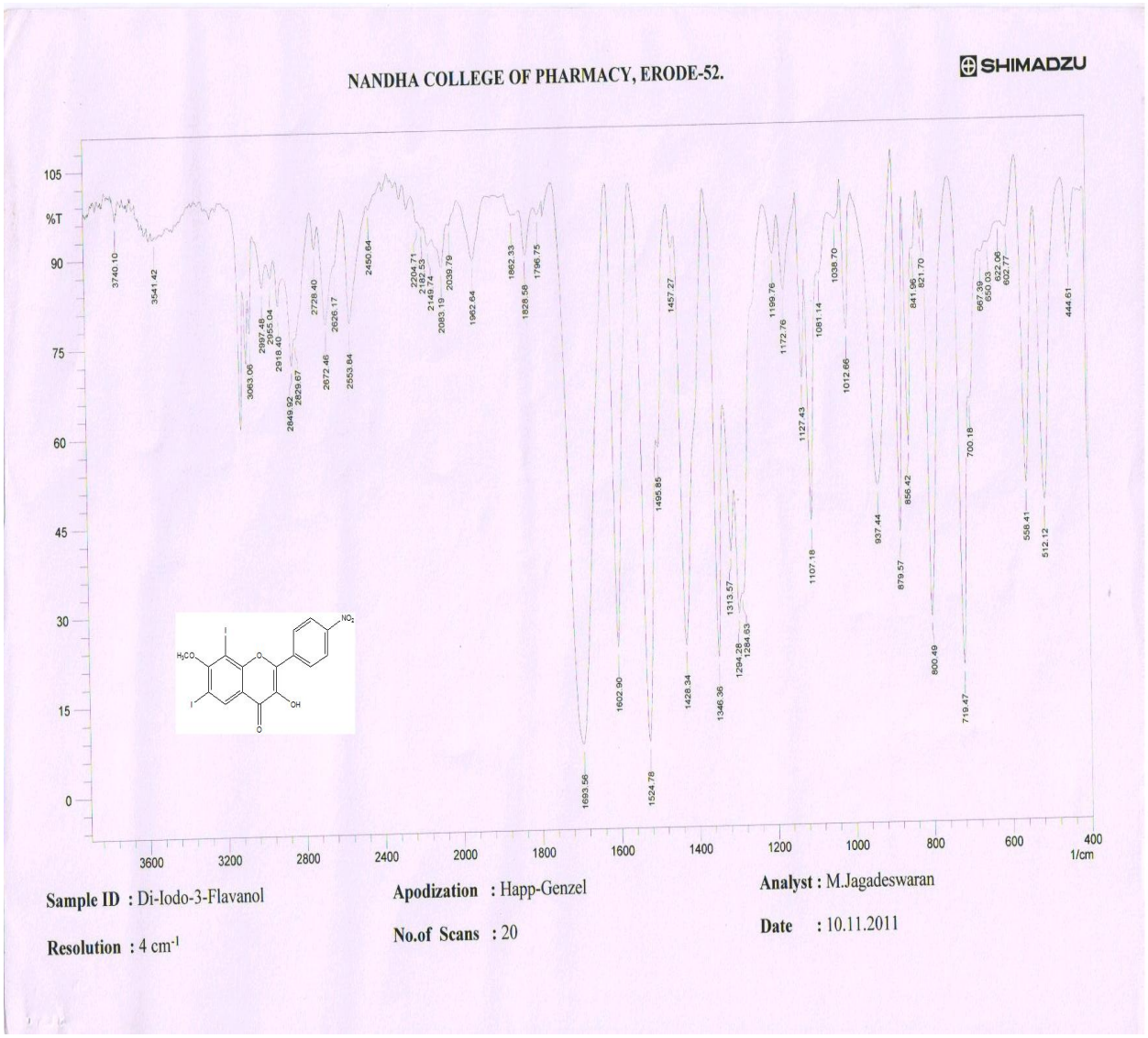

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
100
%T
90
80
70
60
50
40
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
3644.62
3445.94
3214.48
2950.22
2892.36
2357.09
2046.54
1868.12
1767.82
1727.31
1719.60
1639.55
1605.79
1563.36
1511.28
1485.24
1461.13
1440.87
1423.51
1420.05
1398.87
1367.58
1343.46
1303.92
1275.95
1252.81
1203.62
1176.62
1159.26
1145.40
1129.98
1112.00
1083.07
1028.09
983.73
956.72
933.58
898.86
878.60
827.49
802.41
759.01
716.58
704.04
660.64
636.53
621.10
562.27
542.98
509.22
447.50
433.03
419.53
O
OH
OCH3
Sample ID : Anisaldehyde Final
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 19.12.2011

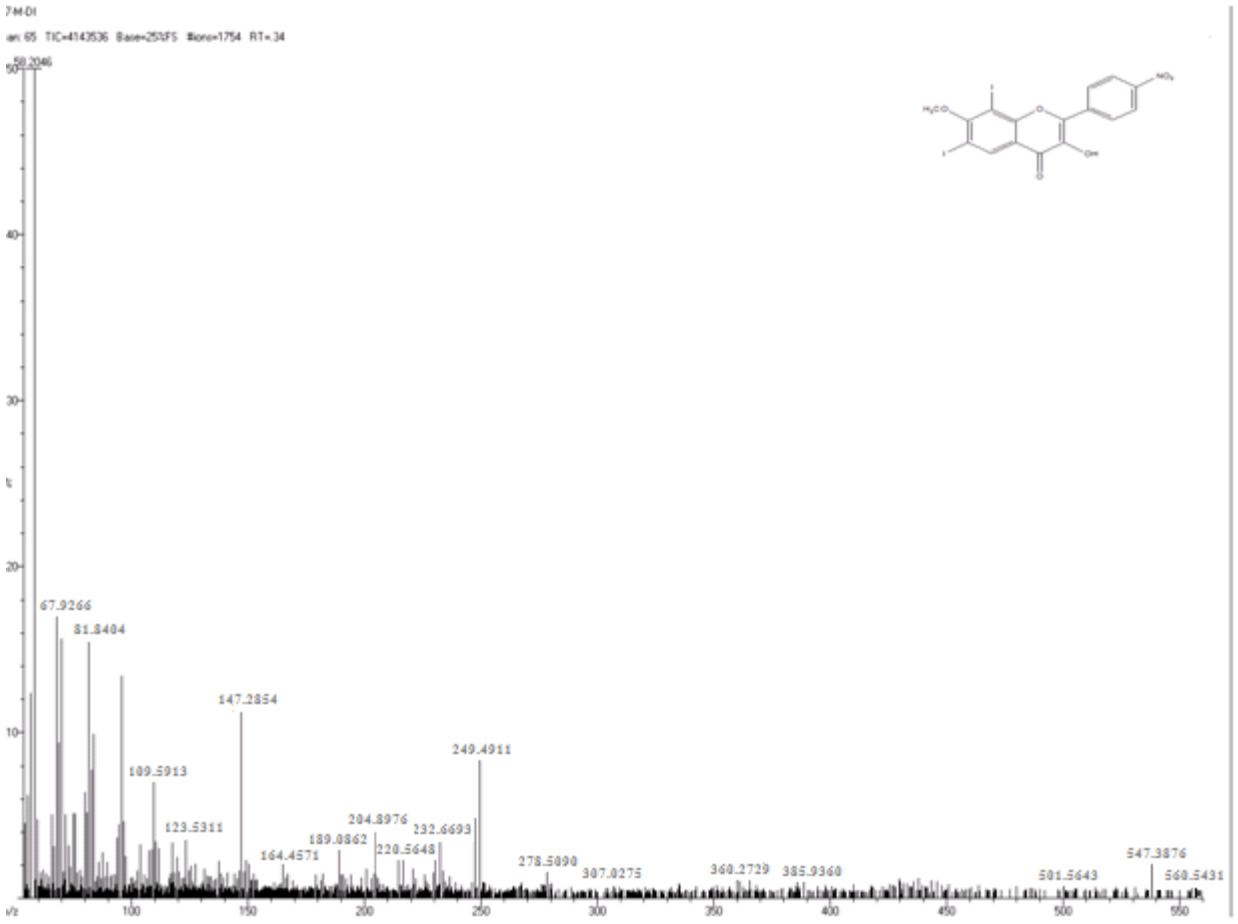

NI-F
Scan: 4 TIC=2891376 Base=3.3%FS #ions=2122 RT=.02
69.8227
100
80
60
%
40
20
103.5124
135.2109
146.9495
89.6304
79.6628
96.5865
118.7018
125.4742
153.7117
161.4577
179.0390
184.3690
197.5310
202.6587
211.5220
220.2907
229.5909
238.8795
249.3303
258.0626
268.9920
m/z
80
100
120
140
160
180
200
220
240
260
OCH3
OH
O
O

COMPOUND F

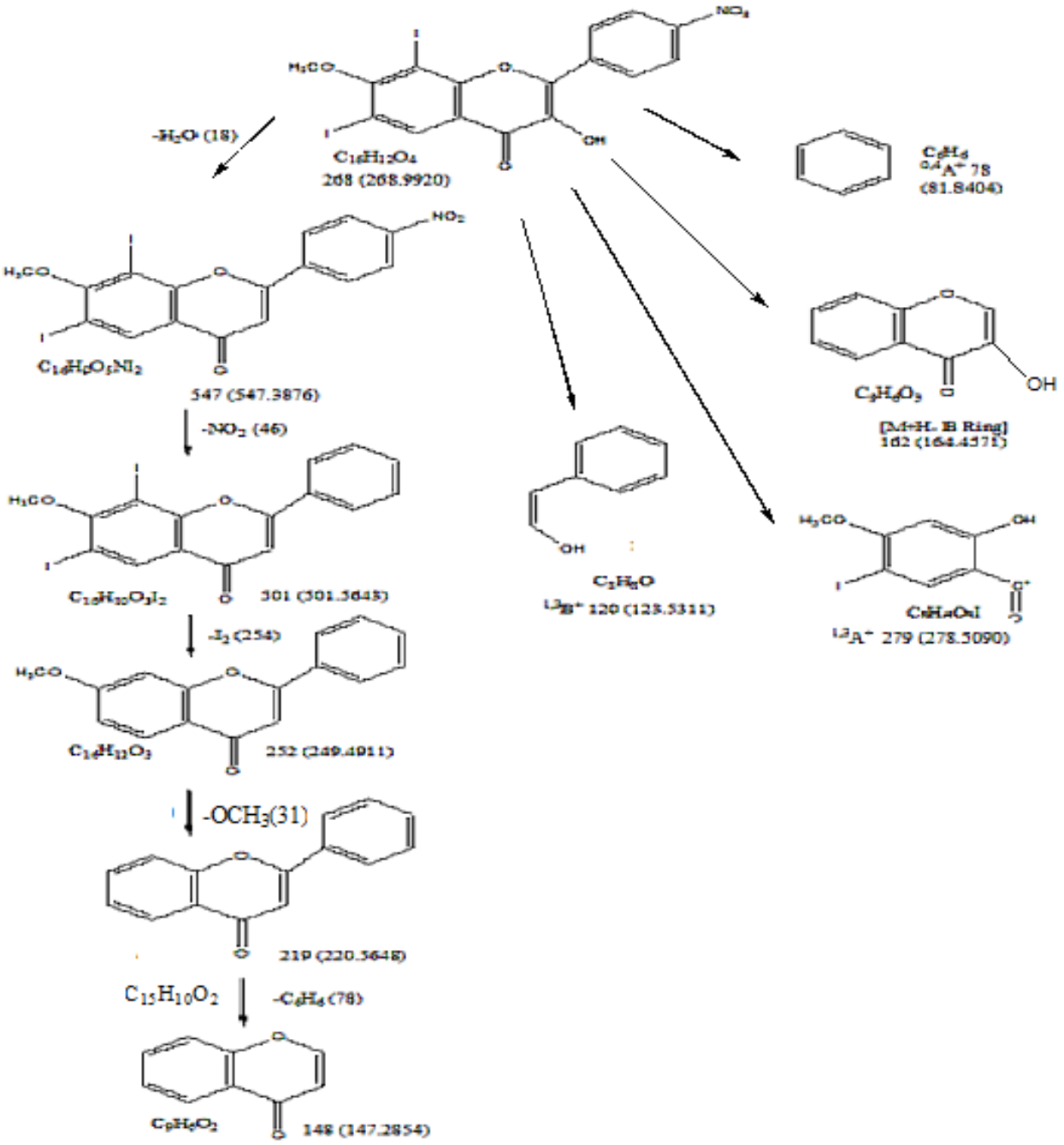

-H2O (18)
C16H12O4
268 (268.9920)
C6H6
0.6A+ 78 (79.6628)
C16H12O3
250 (249.3303)
C9H6O3
[M+H- B Ring] 162 (161.4577)
-OCH3 (31)
C15H10O2
219 (220.2907)
-C6H6(78)
C9H6O2
148 (146.949
C9H6O2
0.2A+ 135 (135.2109)
C15H11O2
1.1A+ 209 (211.5220)
C9H9O
3B+ 120 (118.7018)

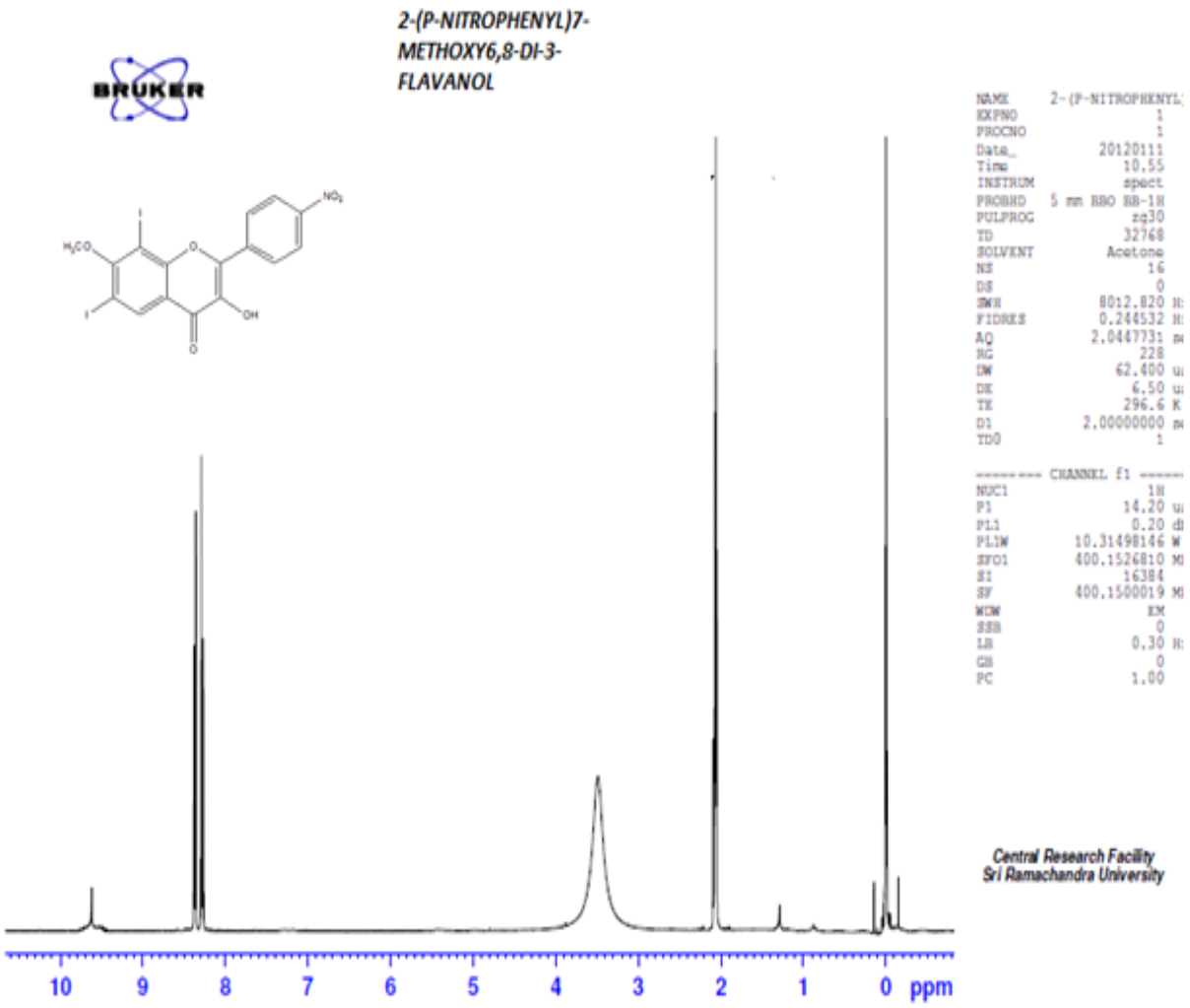

2-(P-METHOXYPHENYL)-3-FLAVONOL
BRUKER
OCH3
OH
O
O
NAME 2(P-METHOXYPHENYL)-
EXPNO 1
PROCNO 1
Date_ 20120111
Time 11.48
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 362
DW 62.400 usec
DE 6.50 usec
TE 296.0 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 14.20 usec
PL1 0.20 dB
PL1W 10.31498146 W
SFO1 400.1526810 MHz
SI 16384
SF 400.1499990 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 ppm

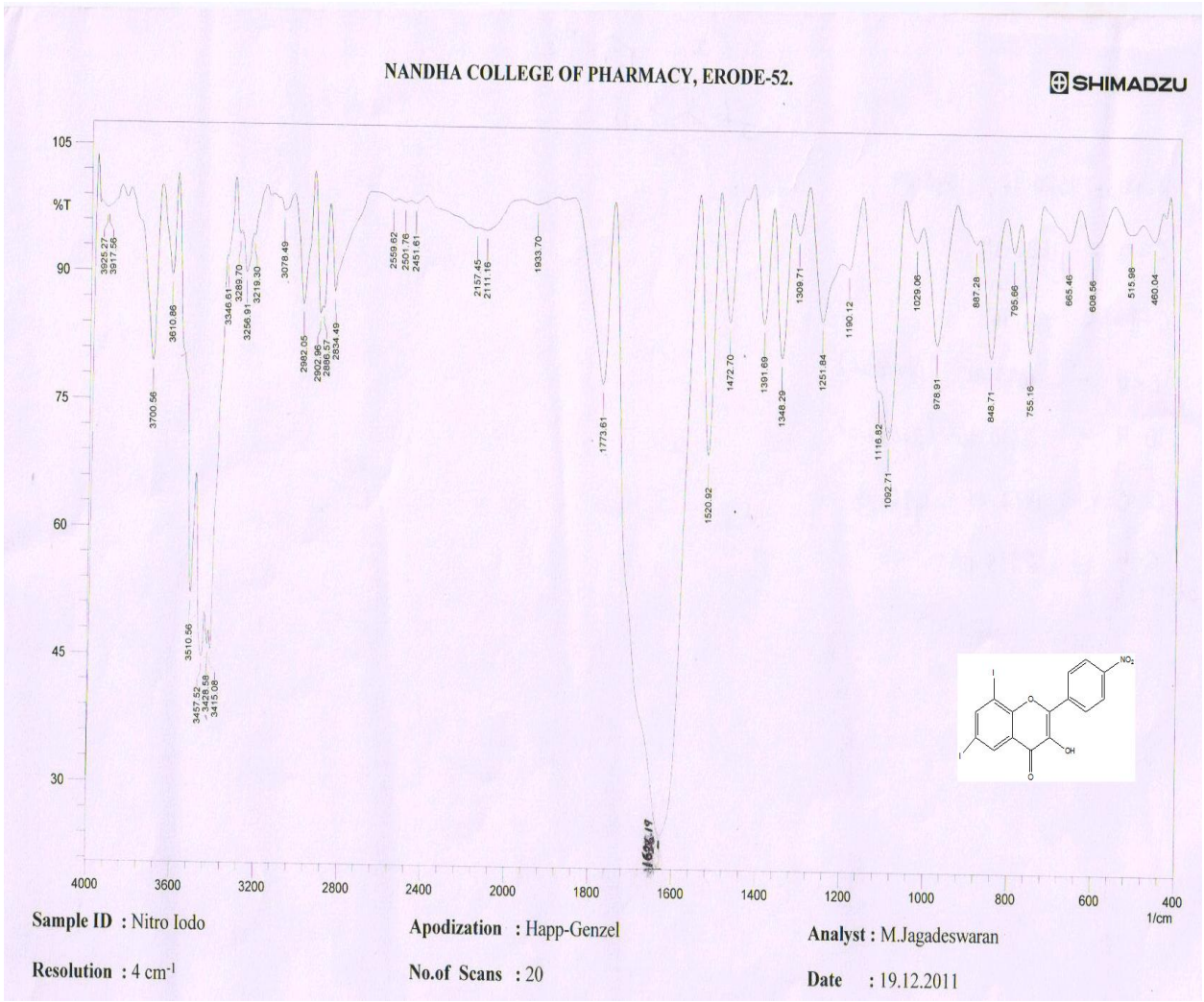

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
%T
1/cm
3740.10
3541.42
3063.06
2957.48
2915.04
2918.40
2849.92
2829.67
2728.40
2672.46
2626.17
2553.84
2450.64
2204.71
2182.53
2149.74
2083.19
2039.79
1962.64
1862.33
1828.58
1796.75
1693.56
1602.90
1524.78
1457.27
1405.85
1428.34
1346.36
1313.57
1284.28
1264.63
1198.76
1172.76
1127.43
1107.18
1081.14
1038.70
1012.66
937.44
879.57
856.42
841.96
821.70
800.49
719.47
700.18
667.39
650.03
622.06
602.77
558.41
512.12
444.61
Sample ID : Di-Iodo-3-Flavanol
Resolution : 4 cm-1
Apodization : Happ-Genzel
No.of Scans : 20
Analyst : M.Jagadeswaran
Date : 10.11.2011

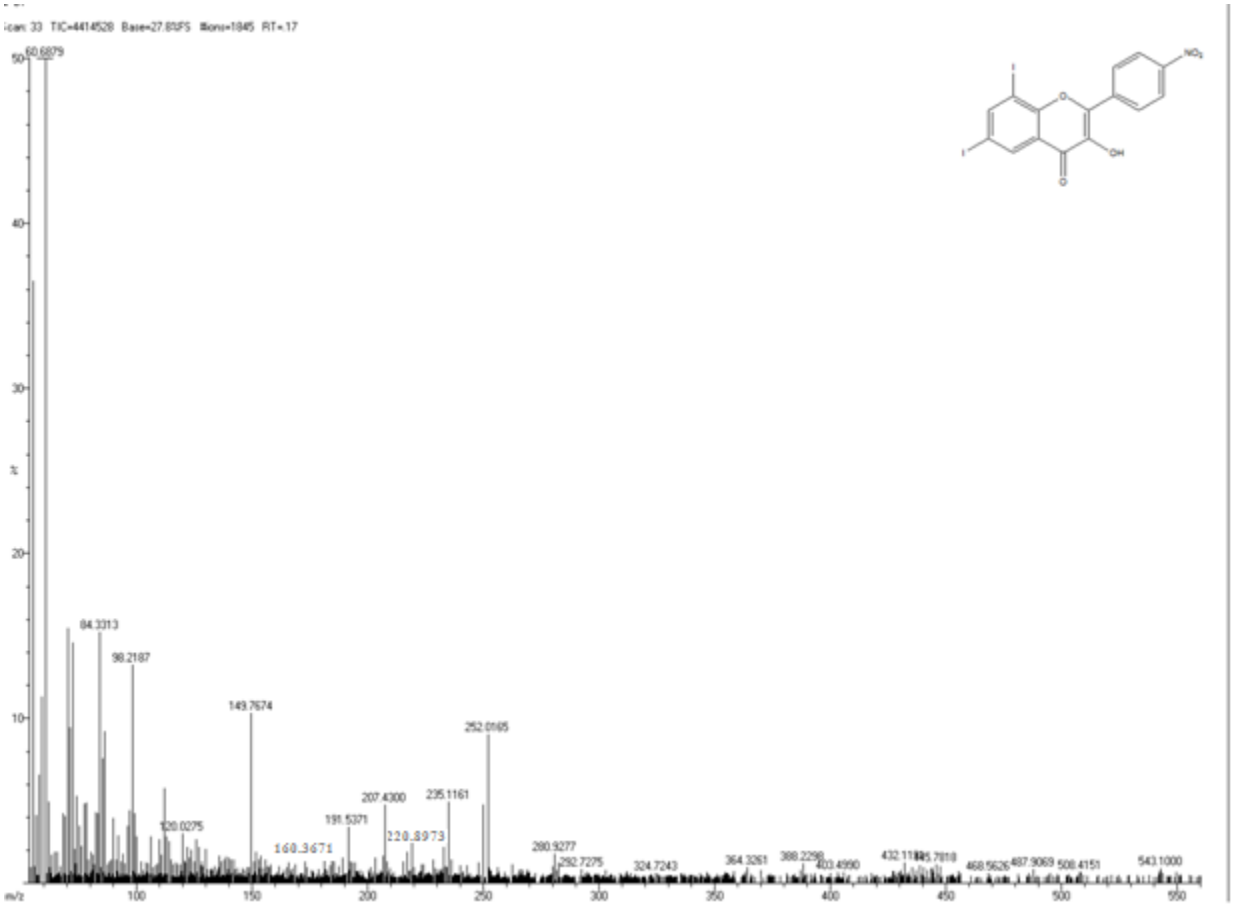

7-M-DI
an 65 TIC=4143536 Base=253FS Ions=1754 RT=.34
50.2046
67.9266
81.8404
109.5913
123.5311
147.2854
164.4571
189.0862
204.8976
220.5648
232.6693
249.4911
278.5090
307.0275
360.2729
385.9360
501.5643
547.3876
560.5431
%
m/z

COMPOUND G

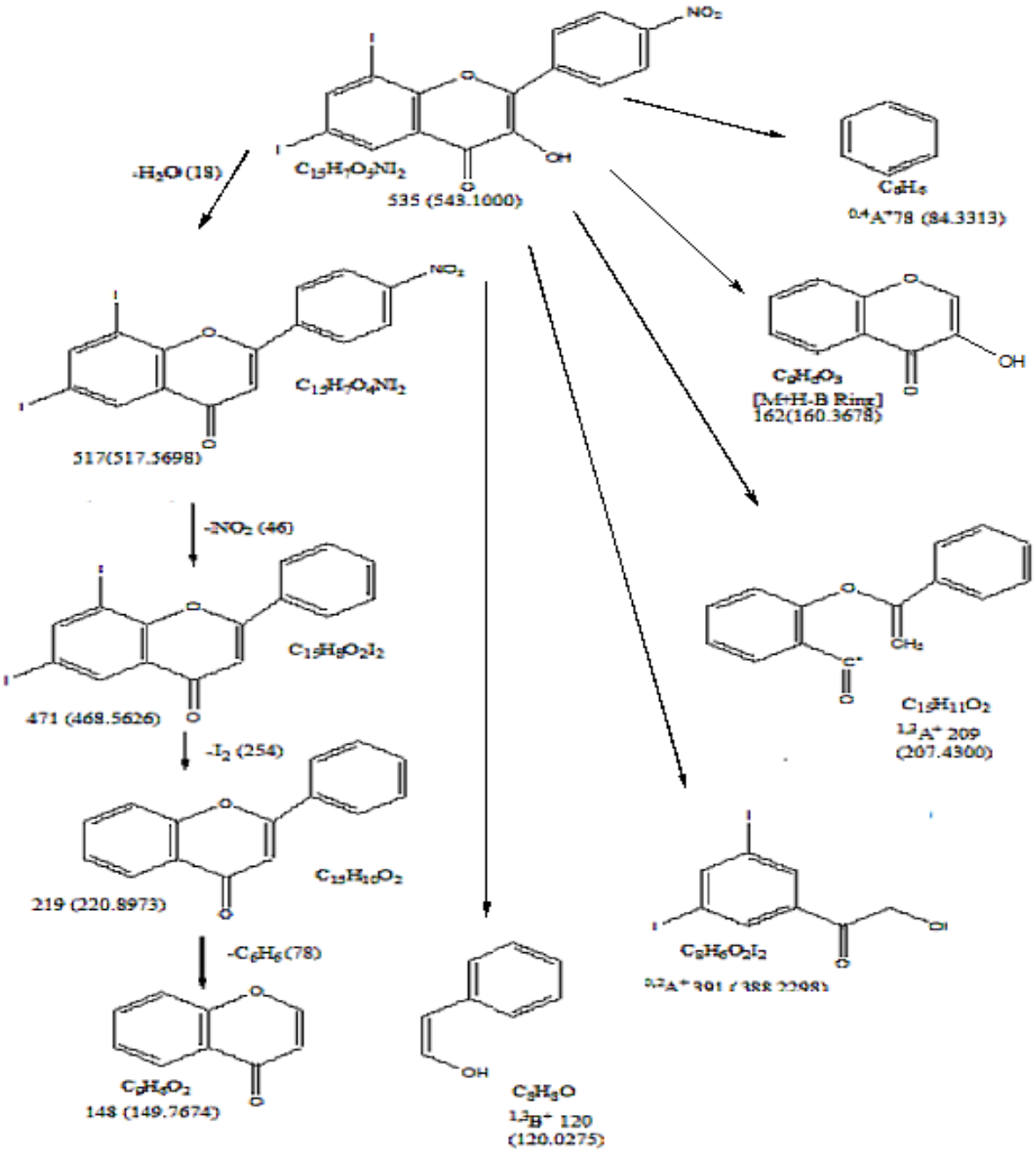

-H2O (18)
C16H12O4
268 (268.9920)
C6H6
0,4A+ 78
(81.8404)
C16H9O5N2
547 (547.3876)
-NO2 (46)
C9H6O3
[A+H- B Ring]
162 (164.4571)
C15H10O3I2
301 (301.3645)
C2H6O
1,3B+ 120 (123.5311)
C8H4OsI
1,3A+ 279 (278.5090)
-I2 (254)
C14H12O3
252 (249.4911)
-OCH3(31)
219 (220.5648)
C15H10O2
-C6H4 (76)
C9H6O2
148 (147.2854)

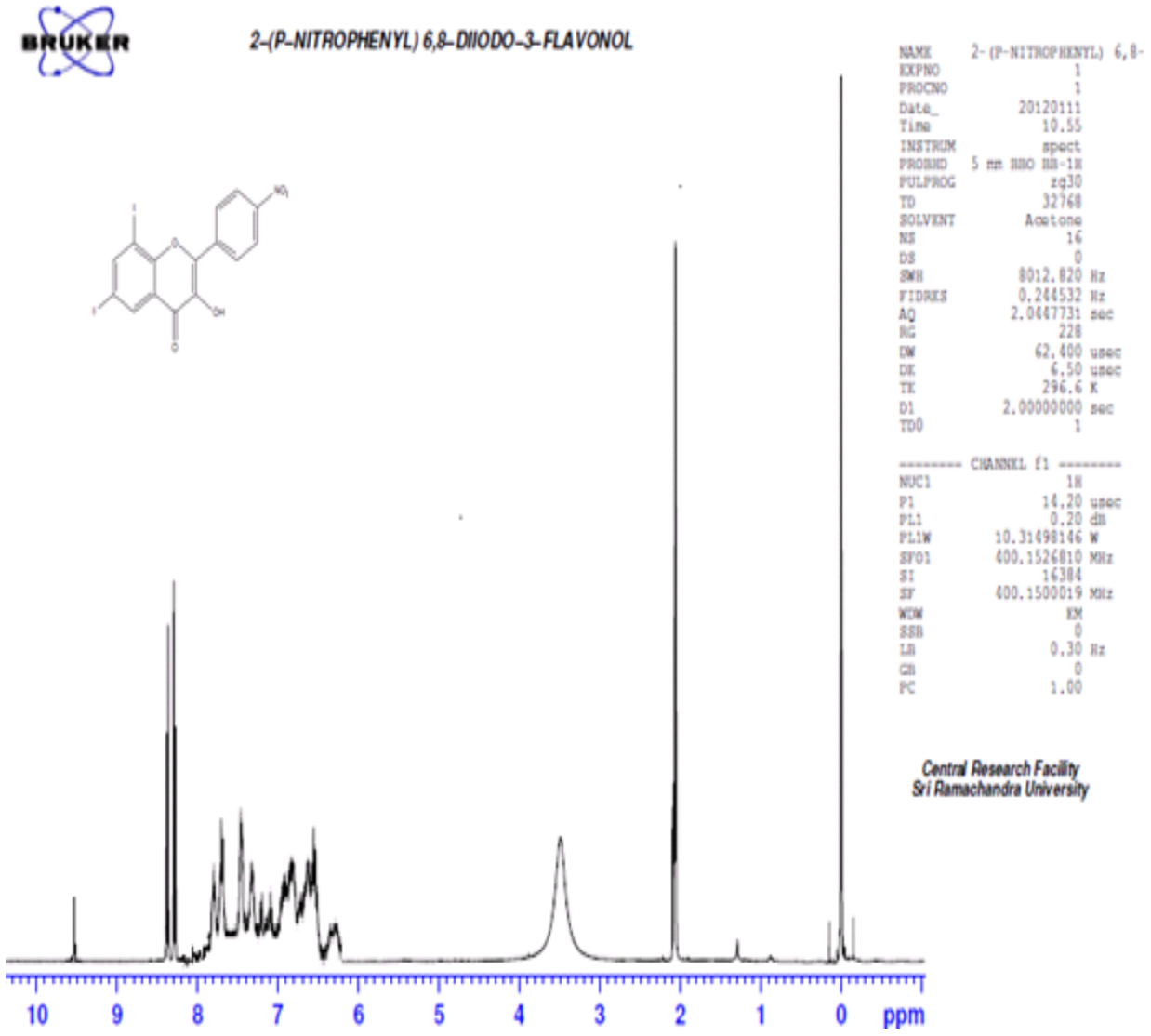

2-(P-NITROPHENYL)7-METHOXY6,8-DI-3-FLAVANOL
BRUKER
NAME 2-(P-NITROPHENYL
EXPNO 1
PROCNO 1
Date_ 20120111
Time 10.55
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 8012.820 H
FIDRES 0.244532 H
AQ 2.0447731 s
RG 228
DW 62.400 u
DE 6.50 u
TE 296.6 K
D1 2.00000000 s
TD0 1
CHANNEL f1
NUC1 1H
P1 14.20 u
PL1 0.20 d
PL1W 10.31498146 W
SFO1 400.1526810 M
SI 16384
SF 400.1500019 M
WDW EM
SSB 0
LB 0.30 H
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
10 9 8 7 6 5 4 3 2 1 0 ppm

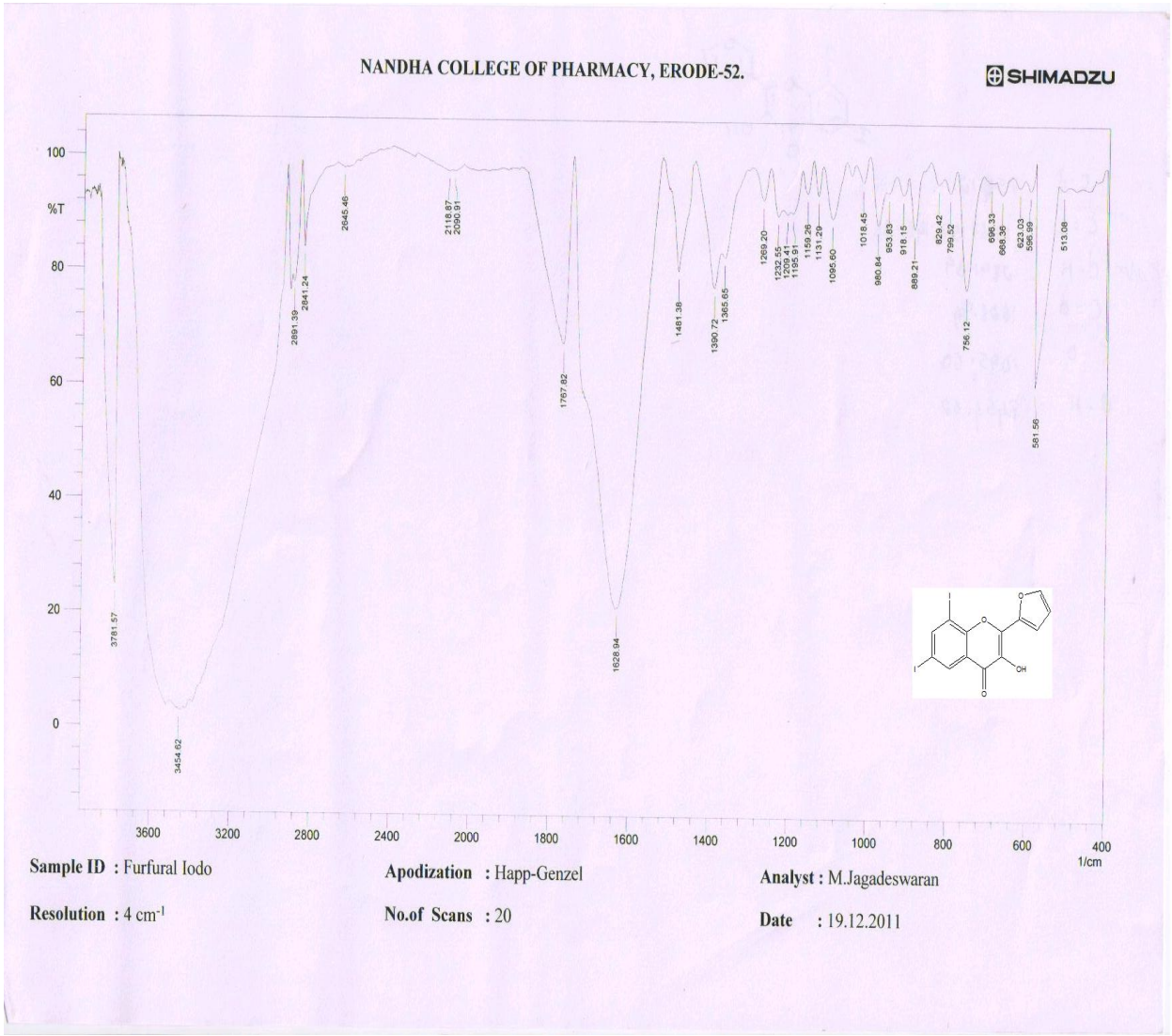

NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
105
%T
90
75
60
45
30
3925.27
3917.56
3700.56
3610.86
3510.56
3457.52
3415.08
3346.61
3289.70
3256.91
3219.30
3078.49
2982.05
2902.96
2869.17
2834.49
2659.82
2501.76
2451.61
2157.45
2111.16
1933.70
1773.61
1520.92
1472.70
1391.69
1348.29
1309.71
1251.84
1190.12
1116.82
1092.71
1029.06
978.91
887.28
848.71
795.66
755.16
665.46
608.56
515.98
460.04
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
Sample ID : Nitro Iodo
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 19.12.2011

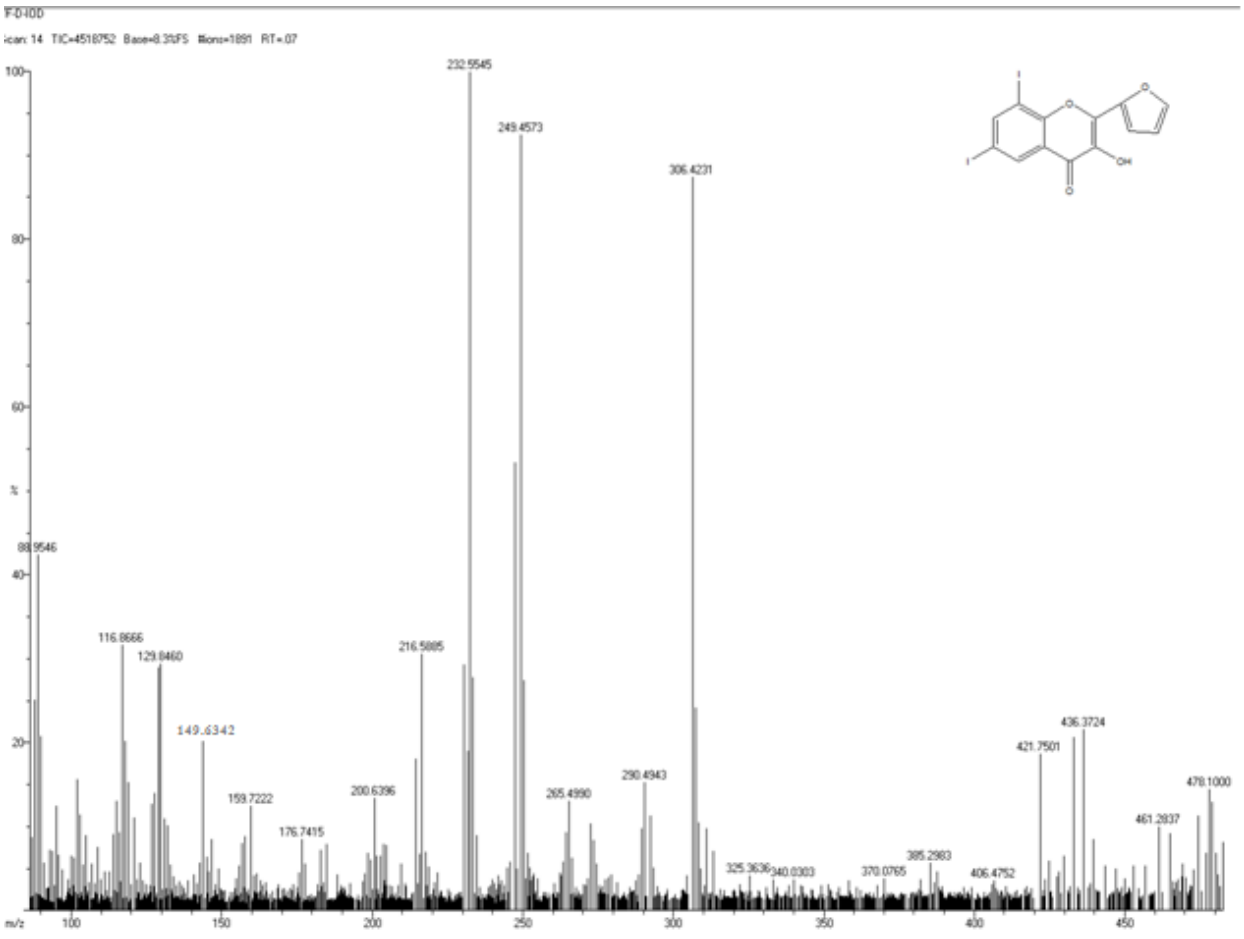

60.6879
84.3313
98.2187
149.7674
252.0165
207.4300
235.1161
191.5371
220.8973
120.0275
160.3671
280.9277
252.7275
324.7243
364.3261
388.2298
403.4990
543.1000
m/z
%

COMPOUND H

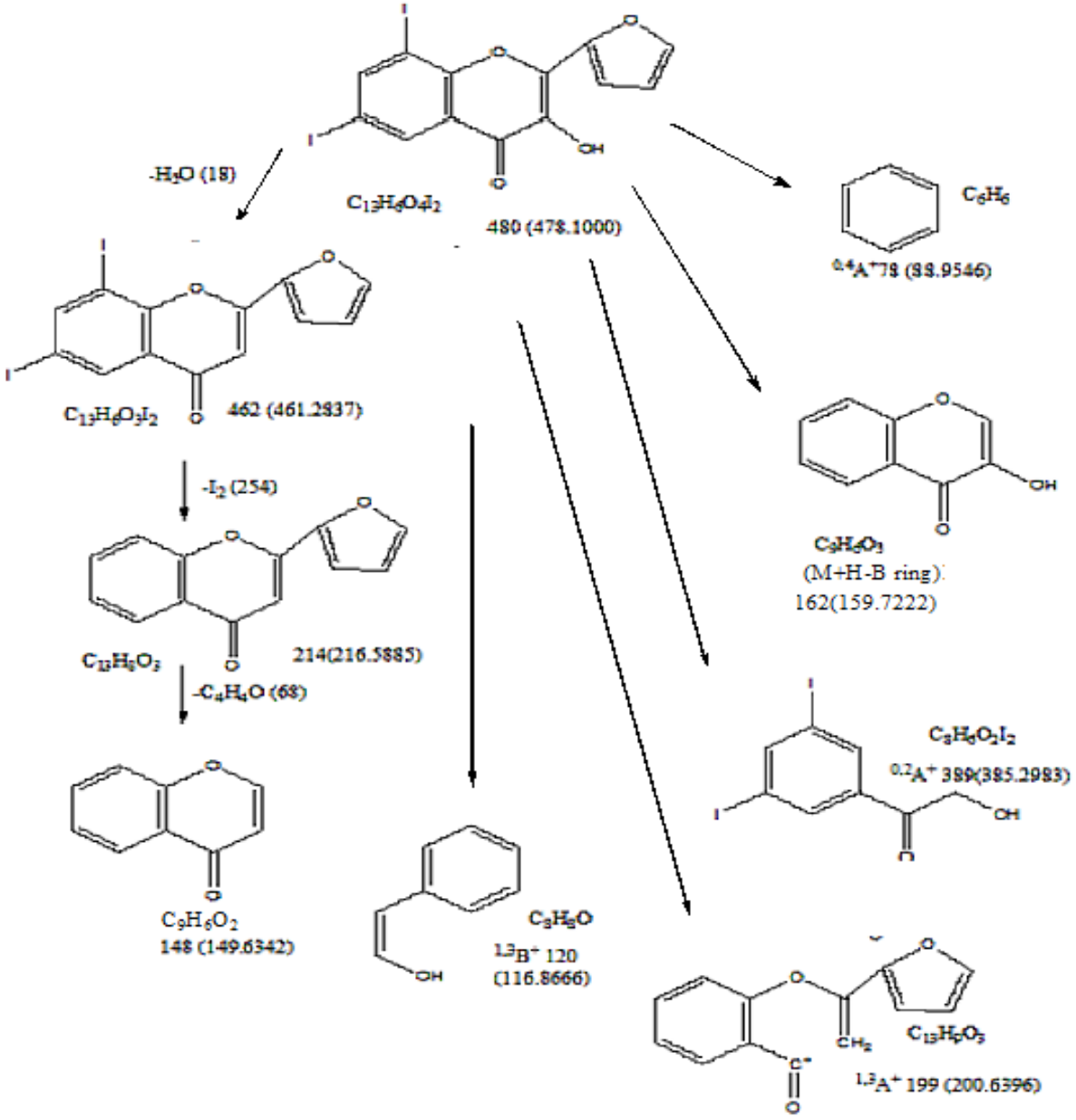

NO2
I
O
OH
-H2O (18)
C15H7O3NI2
535 (543.1000)
C6H5
0.4A+78 (84.3313)
C15H7O4NI2
517(517.3698)
C9H5O3
[M+H-B Ring]
162(160.3678)
-2NO2 (46)
C15H8O2I2
471 (468.5626)
CH2
C+
C15H11O2
1.2A+ 209
(207.4300)
-I2 (254)
C15H16O2
219 (220.8973)
-C6H6(78)
C9H6O2
148 (149.7674)
C3H8O
1.3B+ 120
(120.0275)
C9H6O2I2
0.2A+ 301 (388.2208)

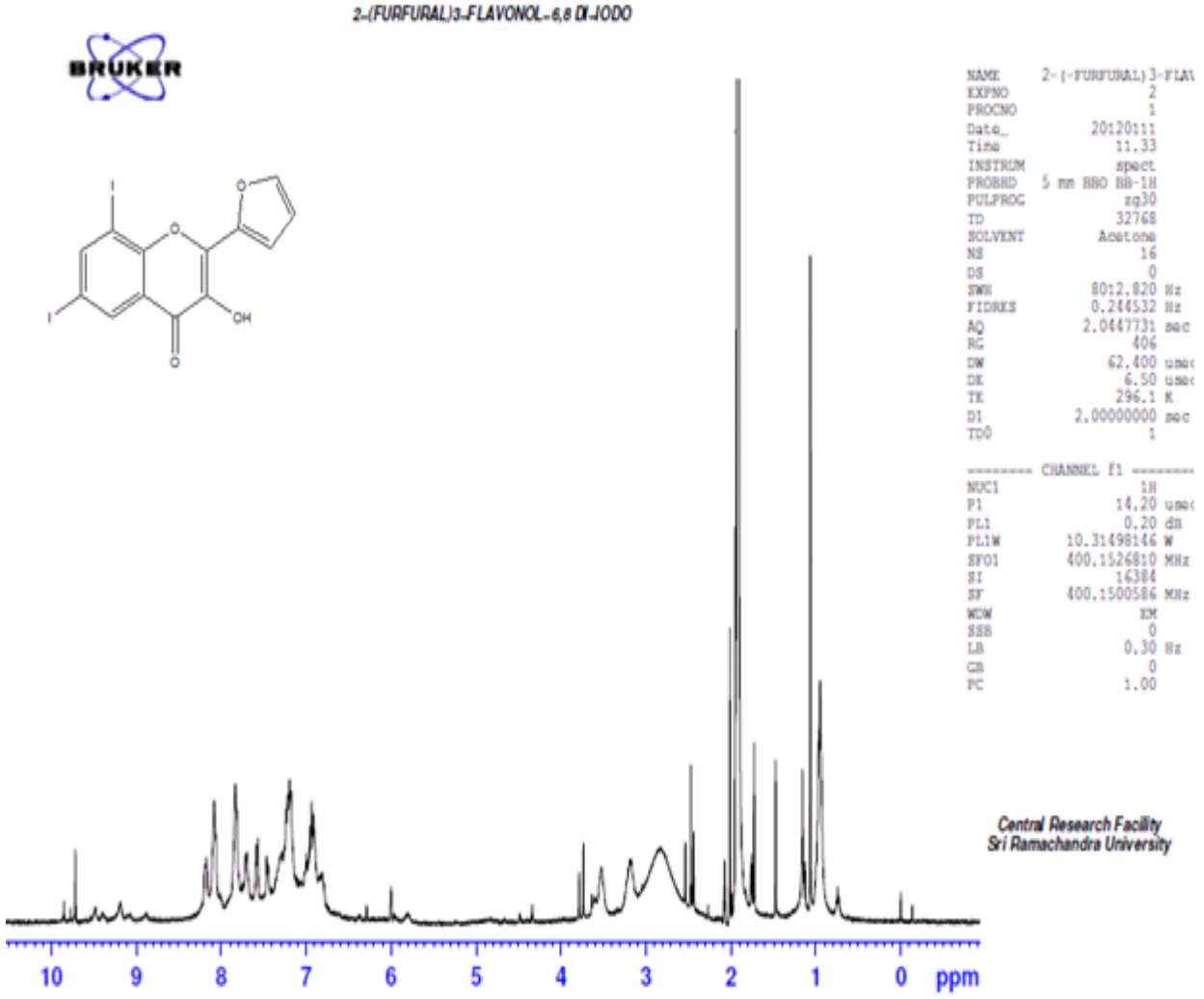

BRUKER
2-(P-NITROPHENYL) 6,8-DIIODO-3-FLAVONOL
NAME 2-(P-NITROPHENYL) 6,8-
EXPNO 1
PROCNO 1
Date_ 20120111
Time 10.55
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 228
DW 62.400 usec
DE 6.50 usec
TE 296.6 K
D1 2.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 14.20 usec
PL1 0.20 dB
PL1W 10.31498146 W
SFO1 400.1526810 MHz
SI 16384
SF 400.1500019 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
10 9 8 7 6 5 4 3 2 1 0 ppm


NANDHA COLLEGE OF PHARMACY, ERODE-52.
SHIMADZU
100
%T
80
60
40
20
0
3781.57
3454.62
2881.39
2841.24
2645.46
2118.87
2090.91
1767.82
1628.94
1481.38
1390.73
1365.65
1269.20
1232.55
1209.41
1195.91
1159.28
1131.29
1095.60
1018.45
980.84
953.83
918.15
889.21
829.42
799.52
756.12
696.33
668.36
623.03
586.89
581.56
513.08
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
Sample ID : Furfural Iodo
Apodization : Happ-Genzel
Analyst : M.Jagadeswaran
Resolution : 4 cm-1
No.of Scans : 20
Date : 19.12.2011

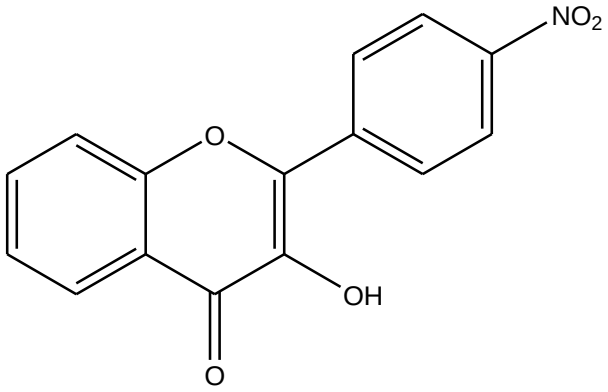

F:\DATA\
Scan: 14 TIC=4518752 Base=8.3\DFS Mono=1891 RT=.07
100
80
60
%
40
20
m/z
100
150
200
250
300
350
400
450
232.5545
249.4573
306.4231
88.9546
116.8666
129.8460
149.6342
159.7222
176.7415
200.6396
216.5885
265.4990
290.4943
325.3636
340.0303
370.0765
385.2983
406.4752
421.7501
436.3724
461.2837
478.1000
I
O
O
OH
I
O

COMPOUND I

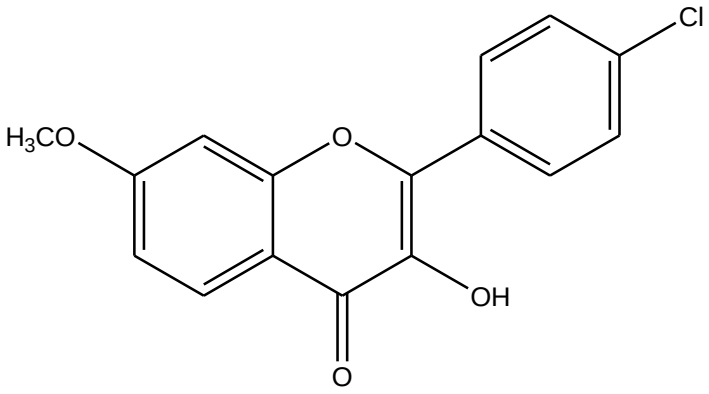

-H2O (18)
C13H6O4I2
480 (478.1000)
C6H6
0,4A+78 (88.9546)
C13H6O3I2
462 (461.2837)
-I2 (254)
C13H8O2
214(216.5885)
-C4H4O (68)
C9H5O3
(M+H-B ring)
162(159.7222)
C9H6O2I2
0,2A+ 389(385.2983)
C9H6O2
148 (149.6342)
C3H5O
1,3B+ 120
(116.8666)
C13H9O3
1,3A+ 199 (200.6396)


2-(FURFURAL)3-FLAVONOL-6,8 DI-IODO
BRUKER
I
O
O
I
OH
O
NAME 2-(-FURFURAL)3-FLA\
EXPNO 2
PROCNO 1
Date_ 20120111
Time 11.33
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 8012.820 Hz
FIDRES 0.244532 Hz
AQ 2.0447731 sec
RG 406
DW 62.400 use
DE 6.50 use
TE 296.1 K
D1 2.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 14.20 use
PL1 0.20 dB
PL1W 10.31498146 W
SFO1 400.1526810 MHz
SI 16384
SF 400.1500586 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Central Research Facility
Sri Ramachandra University
10 9 8 7 6 5 4 3 2 1 0 ppm

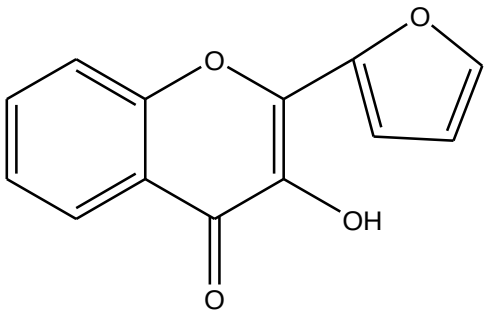
COMPOUND A: 2-(4'-nitrophenyl)-3-hydroxy-4H-Chromen-4-one



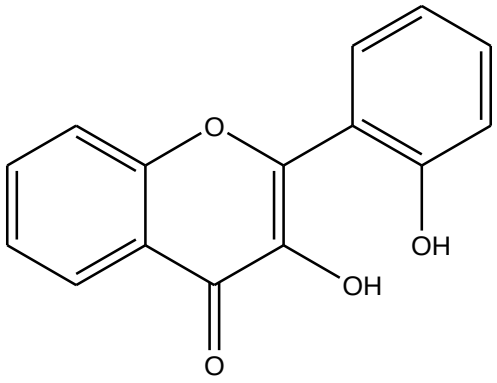
COMPOUND B: 2-(4-chlorophenyl)-7-methoxy-3-hydroxy-4H-Chromen-4-one



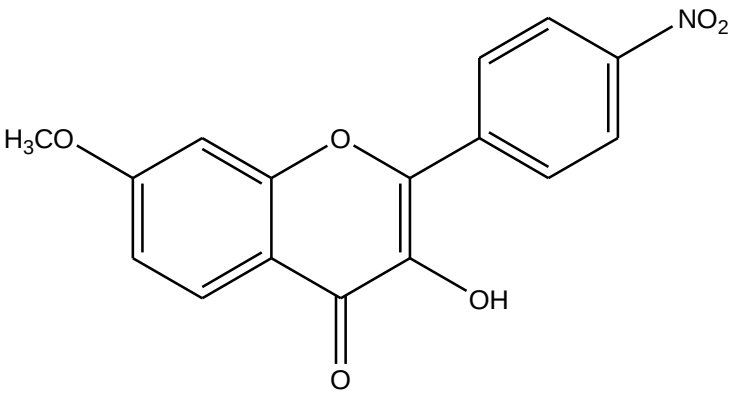
COMPOUND C: 2-(2'-furfural)-3-hydroxy-4H-Chromen-4-one



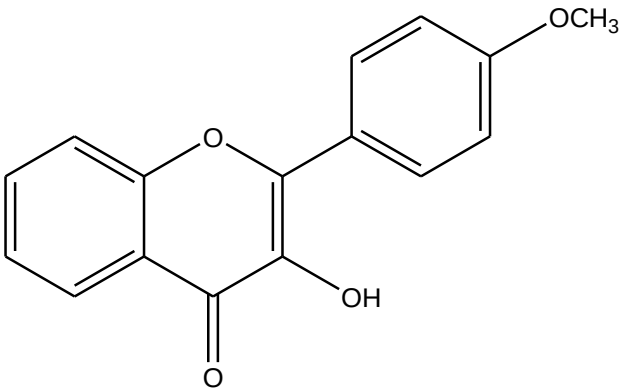
COMPOUND D: 2-(2'-hydroxyphenyl)-3-hydroxy-4H-Chromen-4-one



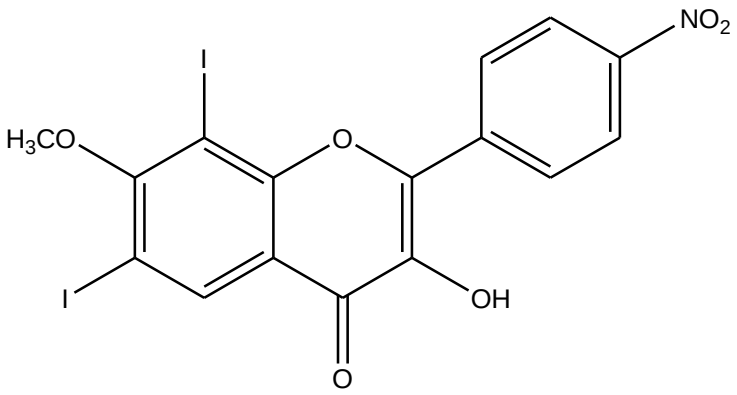
COMPOUND E: 2-(4'-nitrophenyl)-7-methoxy-3-hydroxy-4H-Chromen-4-one



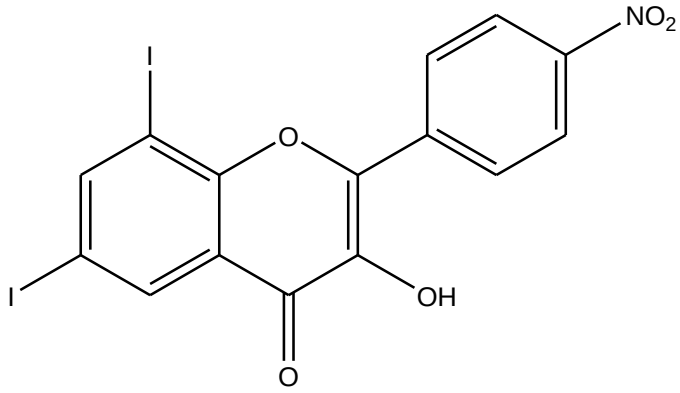
COMPOUND F: 2-(4'-methoxyphenyl)-3-hydroxy-4H-Chromen-4-one



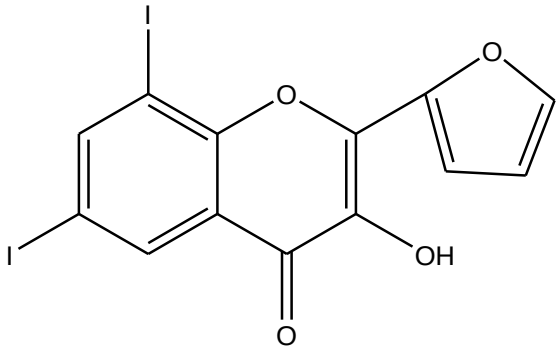
COMPOUND G: 2-(4-nitrophenyl)-6,8-diiodo-3-hydroxy-7-methoxy-4H-Chromen-4-one



COMPOUND H: 2-(4'-nitrophenyl)-3-hydroxy-6,8-diiodo-4H-Chromen-4-one



COMPOUND I: 2-(2'-furfural)-3-hydroxy-6,8-diiodo-4H-Chromen-4-one



7.1 SCREENING OF ANTI-BACTERIAL ACTIVITY

It is evident from the literature that substituted flavonols exhibits pronounced anti-microbial activity. Therefore, it has been felt worthwhile to screen the newly synthesized compounds for their possible anti-bacterial properties.

MATERIAL AND METHODS:

Solutions of all the synthesized compounds were prepared in various concentration using DMSO as solvent. Anti-bacterial evaluation of the synthesized compounds were tested against both gram negative and gram positive bacteria viz., Bacillus subtitles, Bacillus licheniformis, Escherichia coli, Salmonella typhi by using plate hole diffusion method. Streptomycin (1000 µg/ml) is used as a standard for both gram negative and gram positive bacteria. Effectiveness of susceptibility is proportional to the diameter of inhibition of zone was measured.

EXPERIMENTAL DESIGN FOR ANTI-BACTERIAL STUDIES:

Cultivation of Microorganism

The following microorganism was used to study the anti-bacterial activity. Bacterial strains used were maintained at 4^{o}C on nutrient agar plate before use.

1. *Bacillus subtitles* - Gram positive bacteria
2. *Bacillus licheniformis* - Gram positive bacteria
3. *Lacto bacillus* - Gram positive bacteria
4. *Staphylococcus aureus* - Gram positive bacteria
5. *Salmonella typhi* - Gram negative bacteria
6. *Flavobacterium devorans* - Gram negative bacteria
7. *Escherichia coli* - Gram negative bacteria
8. *Klebsiella pneumonia* - Gram negative bacteria

Standard: Streptomycin

Solvent : DMSO

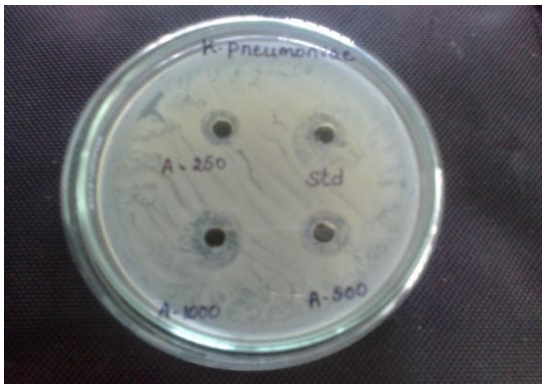
The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water by heating on a water bath and were dispensed in 100 ml volume conical flasks. The conical flasks were closed with cotton plugs and were sterilized by autoclaving at 121^{o}C (15 Ib psig) for minutes. A total of 25 ml of agar medium was poured into sterile universals. The Petridish were specially selected with flat bottom and were placed on level surface so as to ensure that the layer of medium is in uniform thickness. Each universal was inoculated with 0.2 ml of different bacterial strains mixed well with the nutrient agar medium into sterile Petri dishes and then allowed to solidify[30].

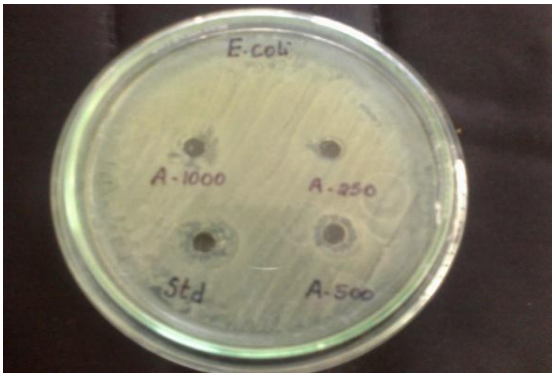
A well was prepared in the plate with help of a cork-borer (6 mm) four holes per plate were made in set of agar containing a bacterial culture. A total of 0.2 ml test solution of synthesized compounds was poured into the wells with the concentrations as 250 µg/ml, 500 µg/ml and 1000 µg/ml were prepared in DMSO as well as standard (1000 µg/ml), using a dropping pipette under aseptic condition and labeled accordingly. The plates were maintained at room temperature for 3-5 hrs to allow diffusion of the solution into the medium. The Petri dishes used for anti-bacterial screening were incubated 37$\pm$ 1^{o}C for 24 hrs. the diameter of zones of inhibition (mm) surrounding each of the wells was recorded. The results were compared to streptomycin (1000 µg/ml) for anti-bacterial activity[30]

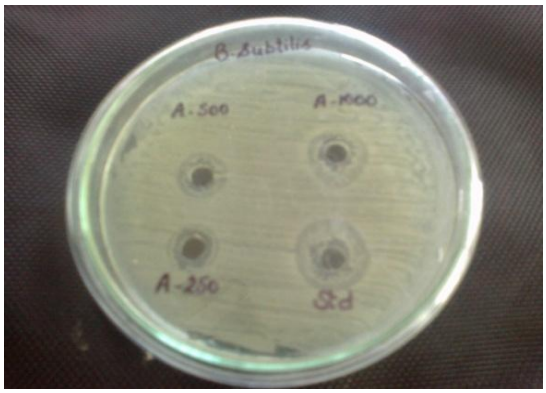
Table No. 5 Screening of Synthesized compounds for Anti-bacterial activity

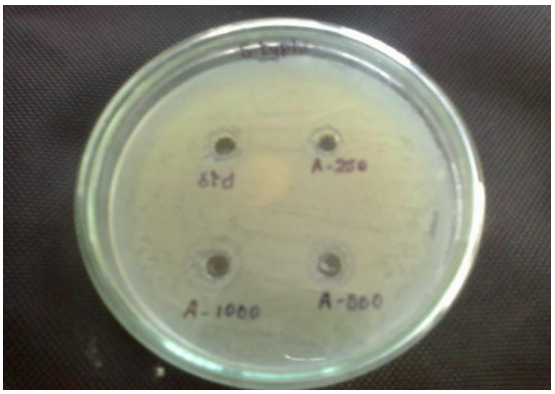
Compounds	Concentration (µg/ml)	Zone of Inhibition (mm)							
		Gram positive bacteria				Gram negative bacteria			
		Bacillus subtilis	*Bacillus licheniformis*	*Lacto bacillus*	*Staphylococcus aureus*	*Salmonella typhi*	*Flavobacterium devorans*	*Escherichia coli*	*Klebsiella pneumonia*
A	500	14	13	13	12	14	15	14	16
	Std	23	22	20	22	23	24	21	22
B	500	17	15	13	14	13	17	15	18
	Std	25	23	20	22	24	25	22	24
C	500	16	14	17	15	14	16	14	16
	Std	23	22	21	22	22	24	21	21
D	500	15	13	16	14	12	17	16	17
	Std	23	21	22	21	21	23	22	23
E	500	17	16	15	13	15	18	17	16
	Std	24	21	23	23	22	23	20	22
F	500	18	15	16	13	15	16	16	17
	Std	24	20	21	22	21	22	22	23
G	500	19	16	15	14	16	17	17	16
	Std	23	21	22	21	20	24	21	23
H	500	18	17	16	16	16	15	16	16
	Std	22	22	21	22	21	22	22	22
I	500	18	15	17	15	14	16	15	17
	Std	23	22	22	22	20	21	20	23

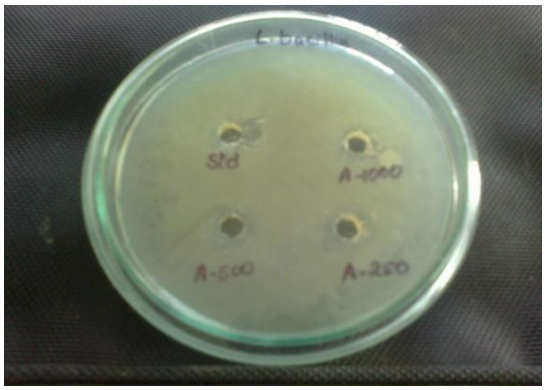
A) 2-(4'-nitrophenyl)-3-hydroxy-4H-Chromen-4- one

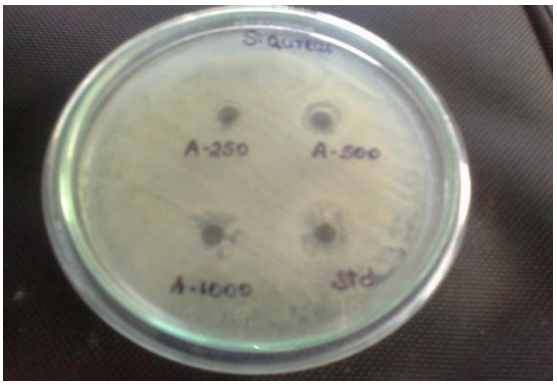


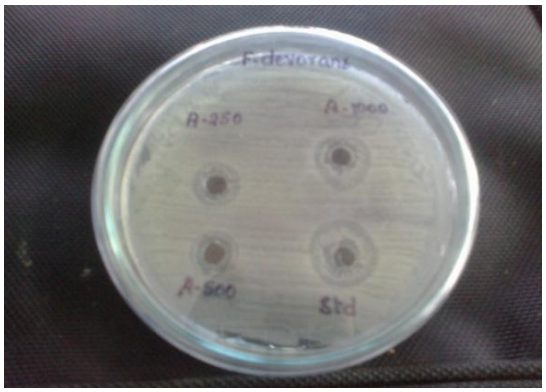


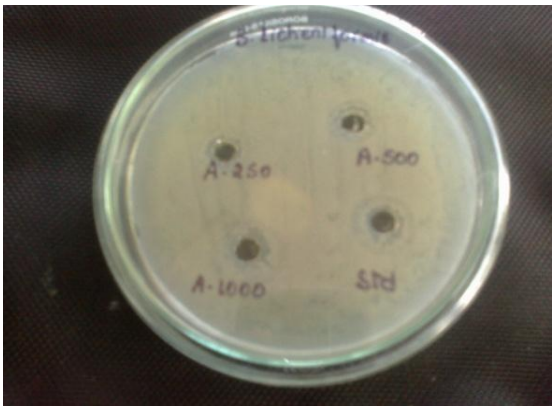




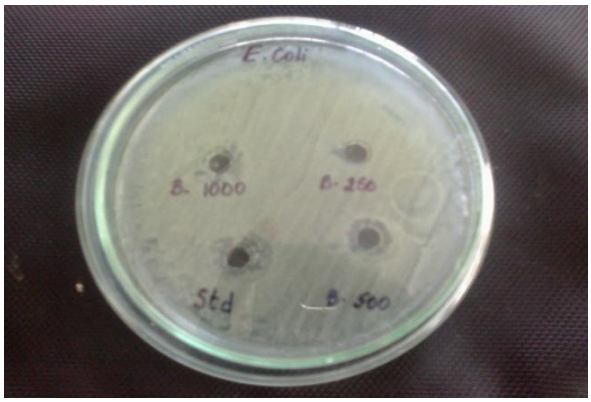


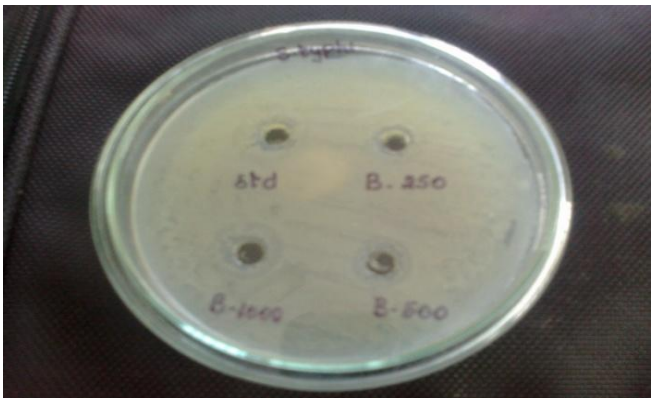


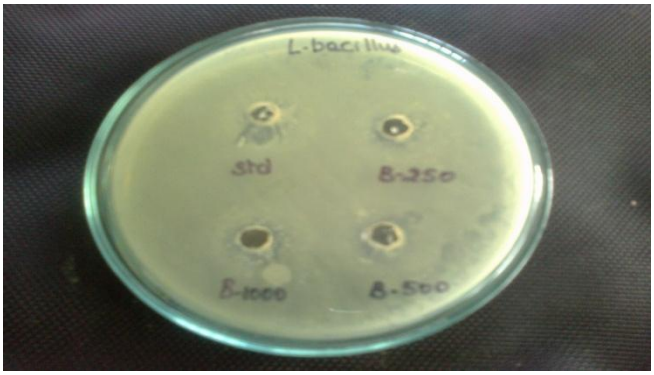


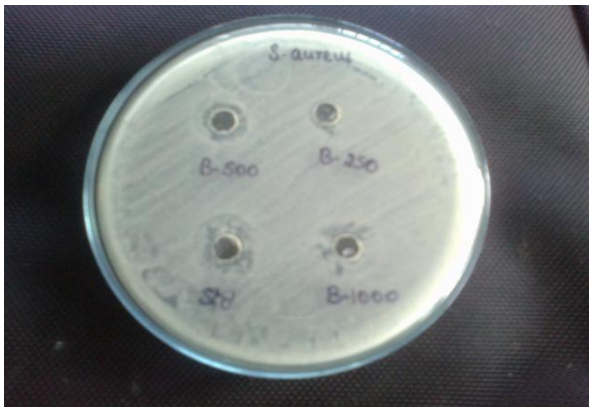


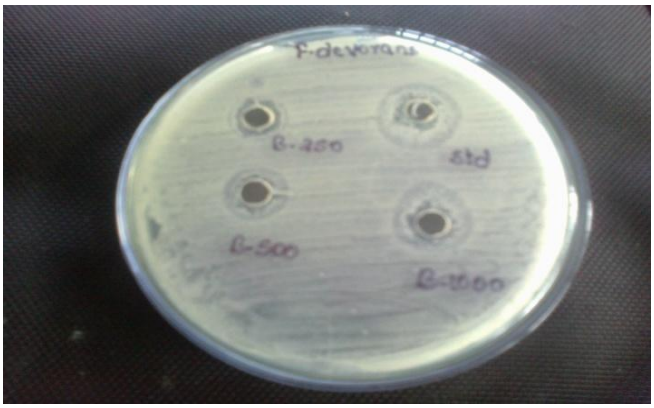
B). 2- (4-chlorophenyl)-7-methoxy-3-hydroxy -4H-Chromen-4-one

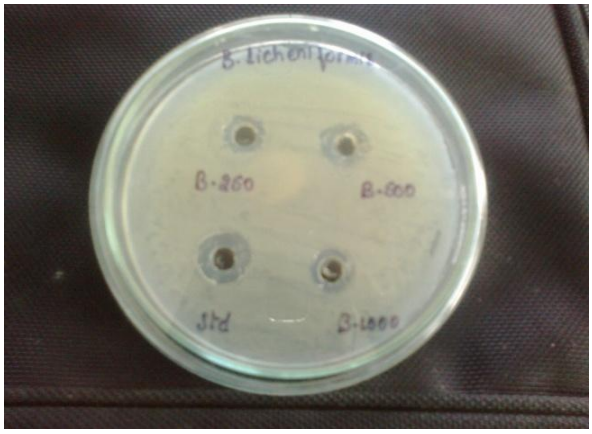


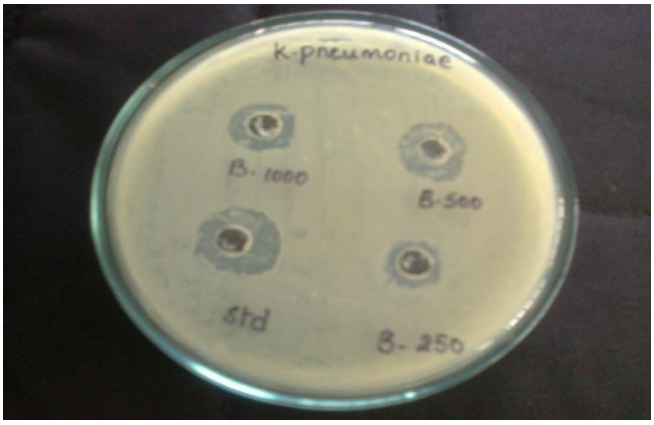




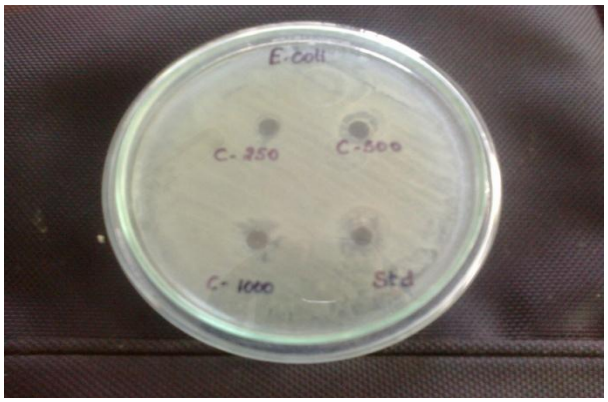


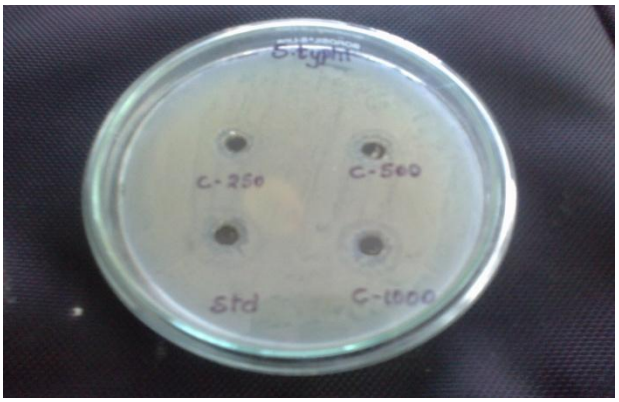


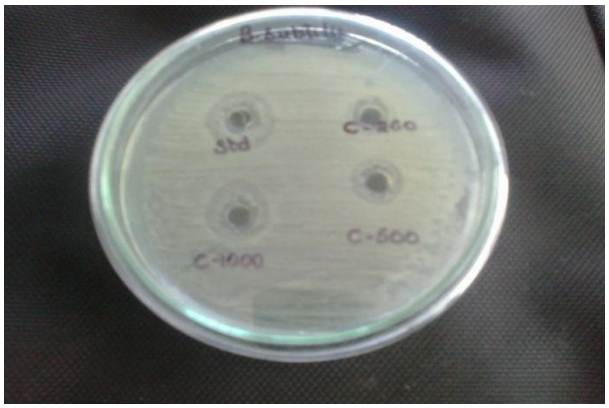


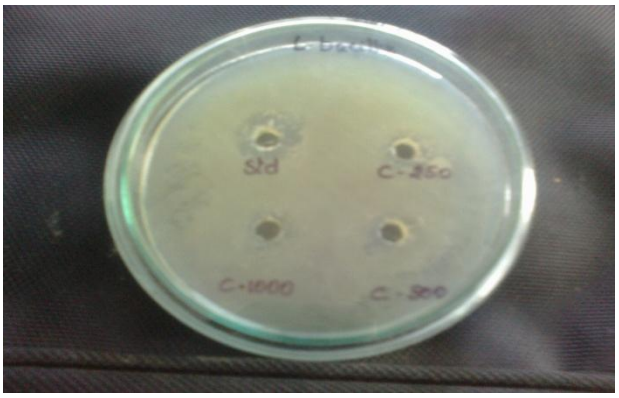


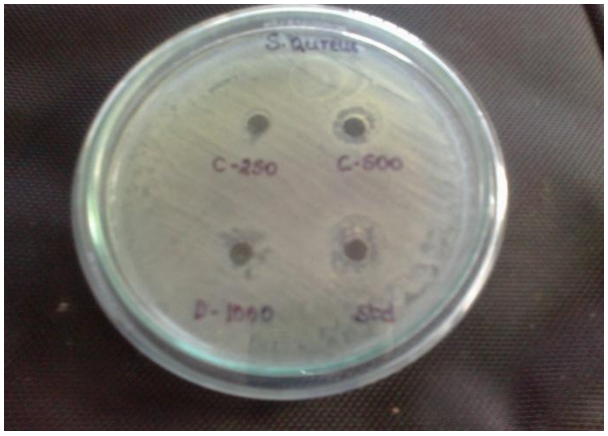
C) 2-(2'-furfural)-3-hydroxy- 4 H-Chromen4-one

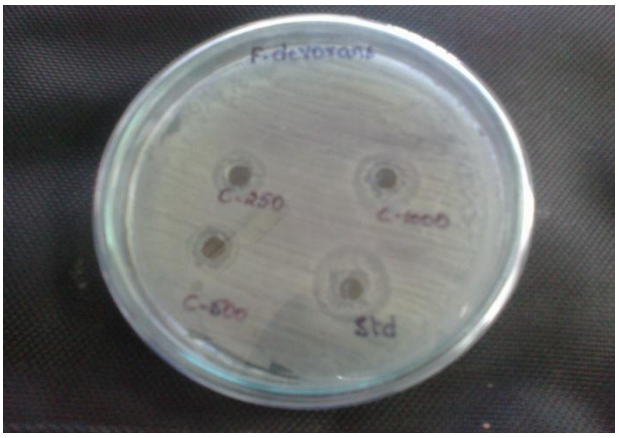


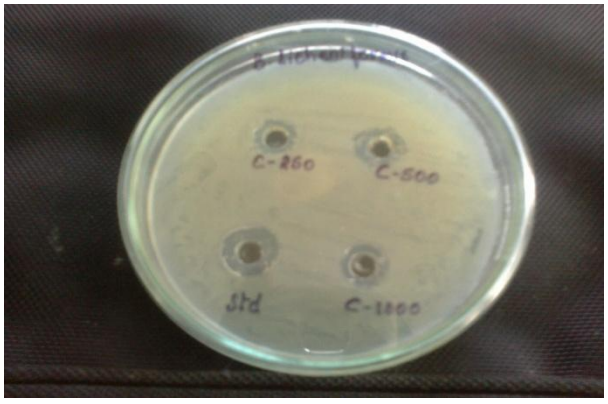


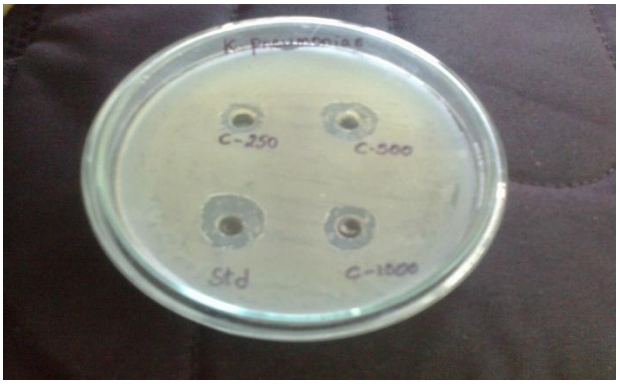




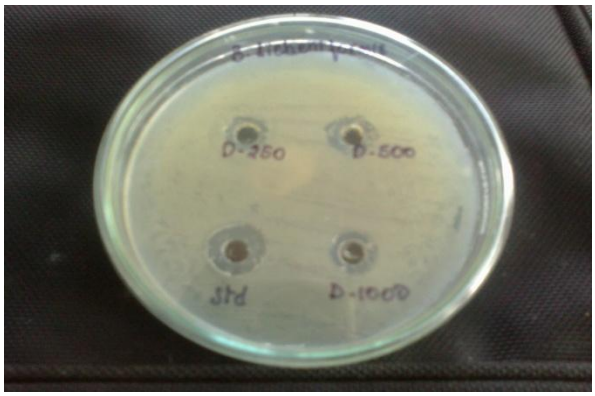


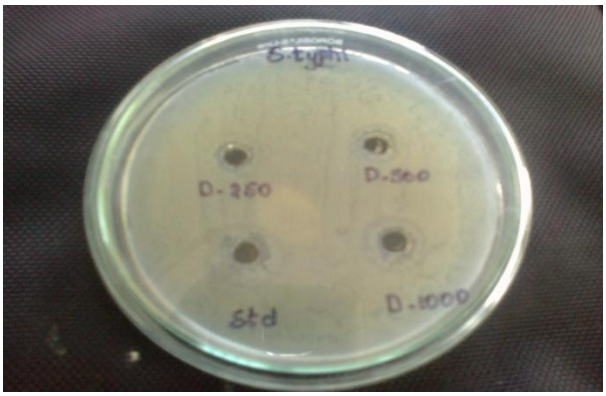


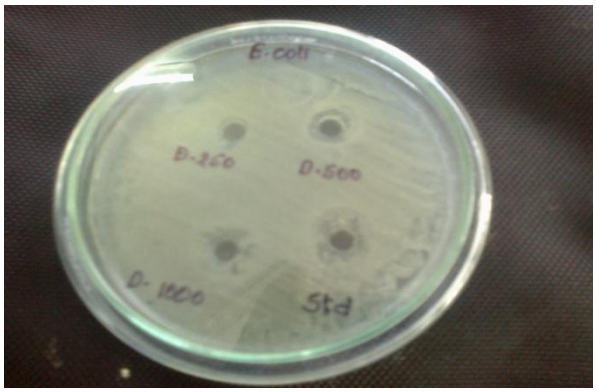


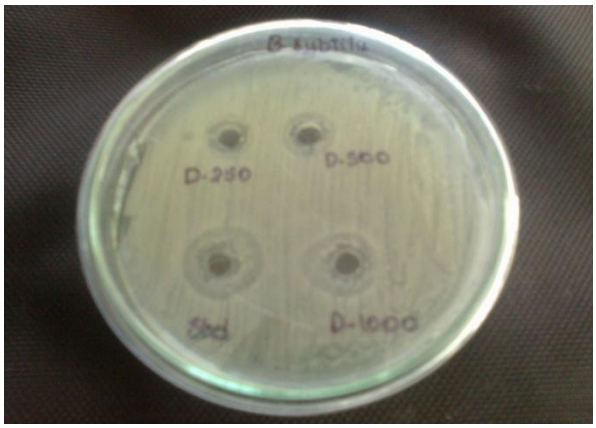


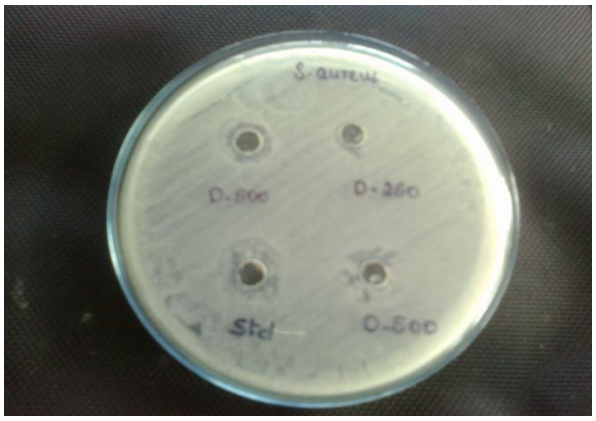
D) 2- (2'-hydroxyphenyl) -3- hydroxy- 4 H-Chromen-4-one

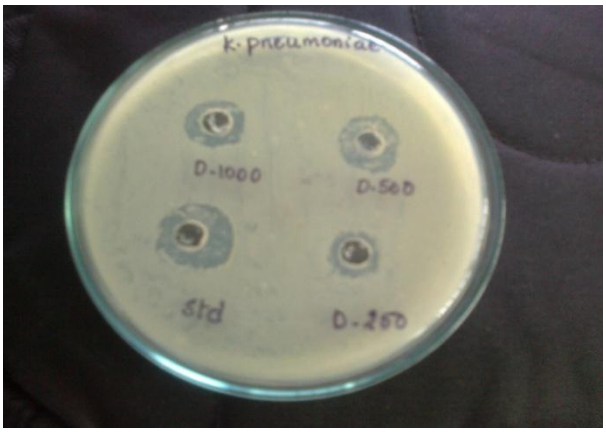




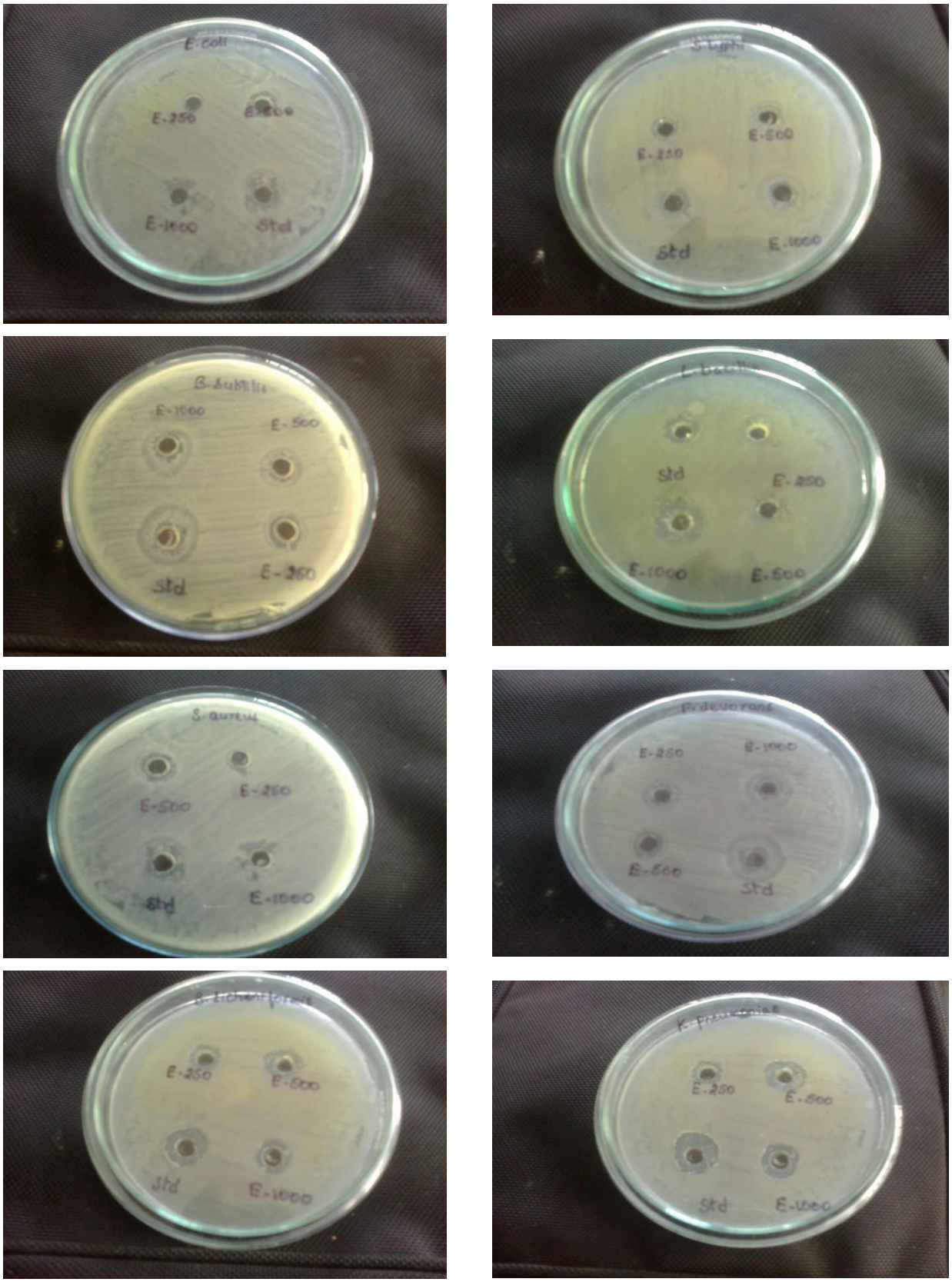




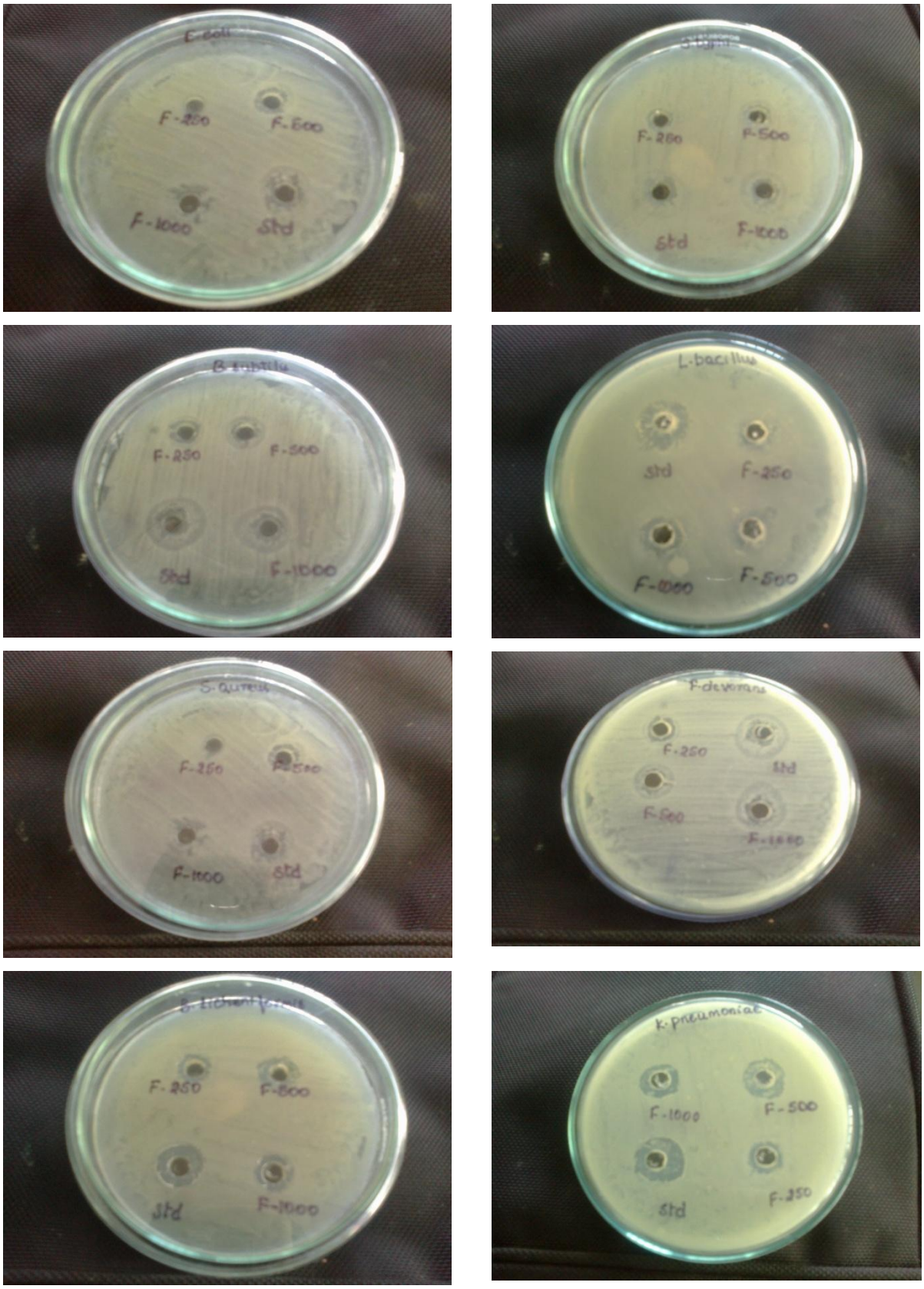




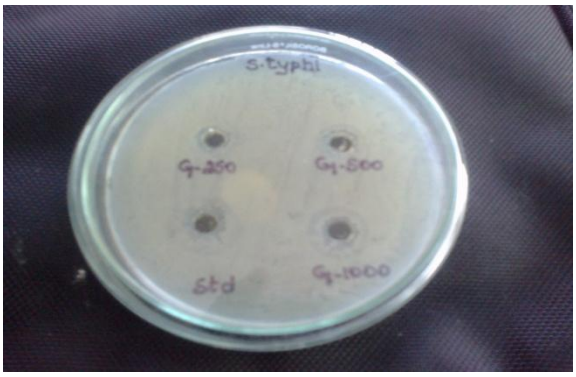
E) 2- (4'-nitrophenyl)- 7-methoxy-3-hydroxy- 4 H-Chromen-4-one

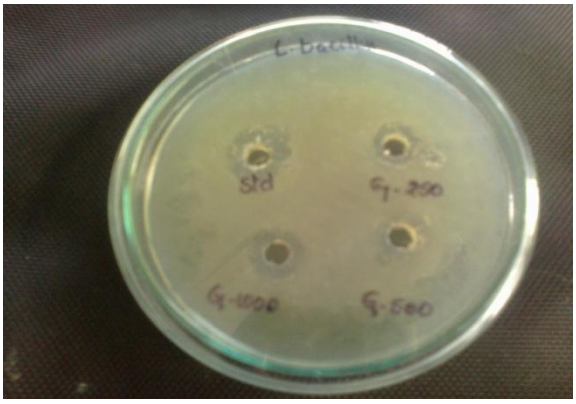


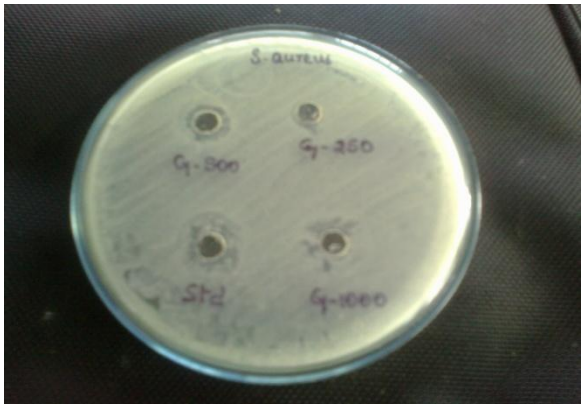
F) 2 - (4'-methoxyphenyl) - 3-hydroxy - 4 H - Chromen-4-one

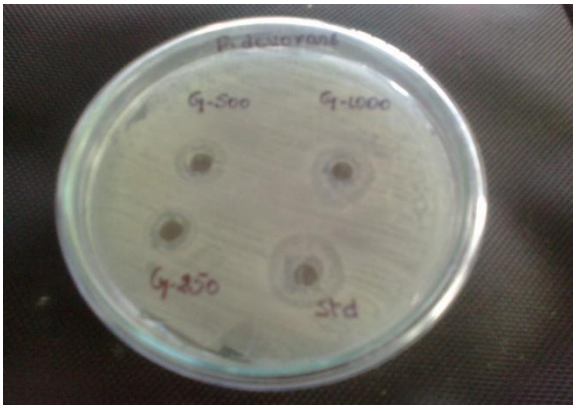


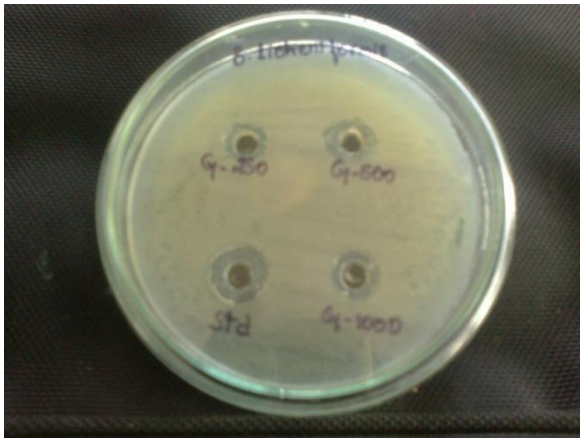
G) 2- (4-nitrophenyl)-6,8-diiodo-3-hydroxy-7- methoxy- 4 H- Chromen-4-one

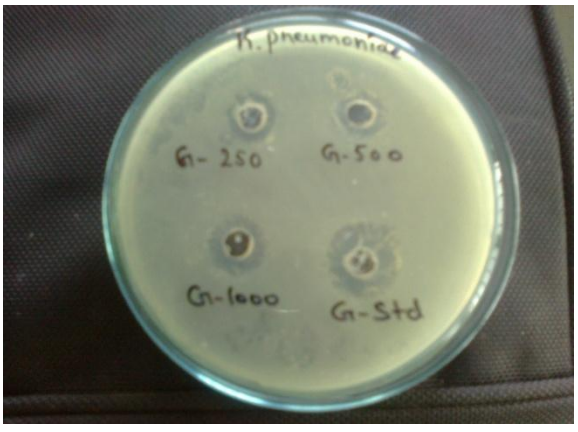




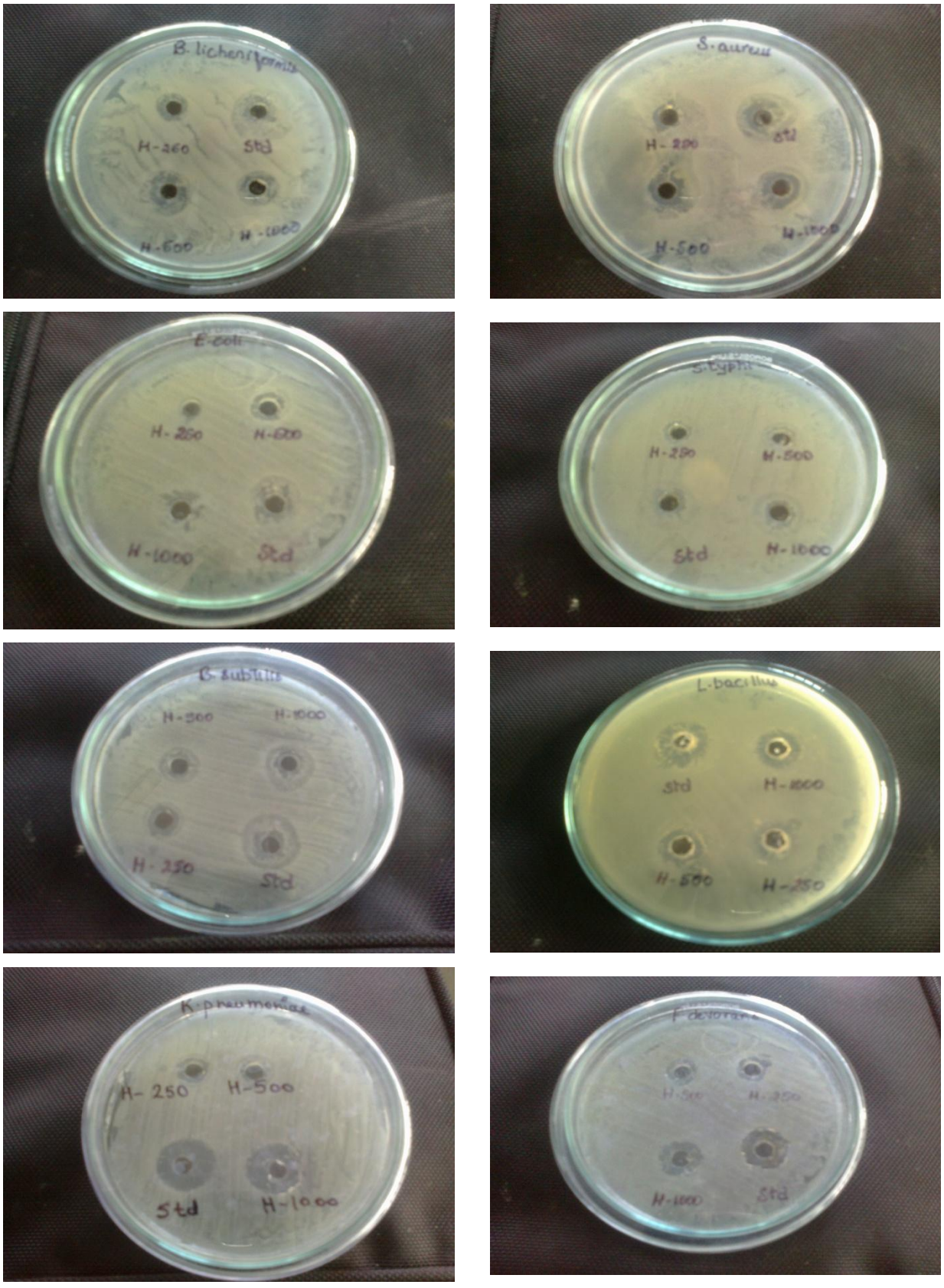




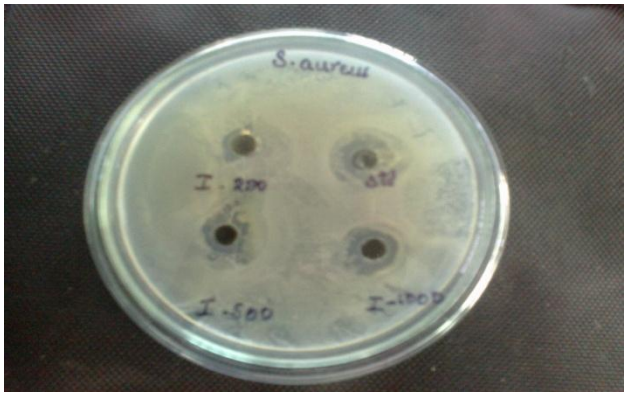


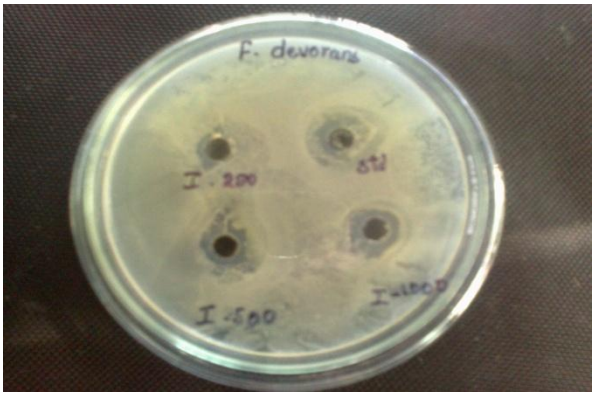


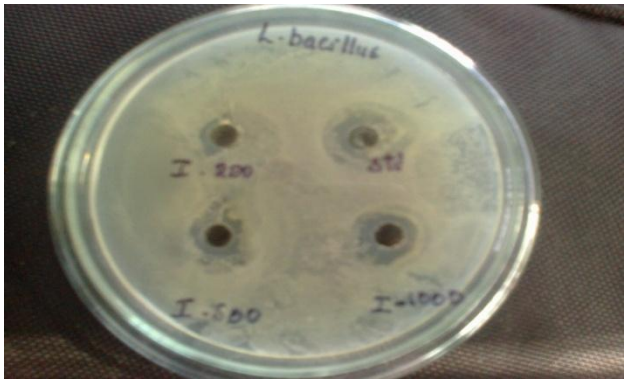
H) 2- (4'-nitrophenyl) -3-hydroxy- 6,8-diiodo- 4 H-Chromen-4-one

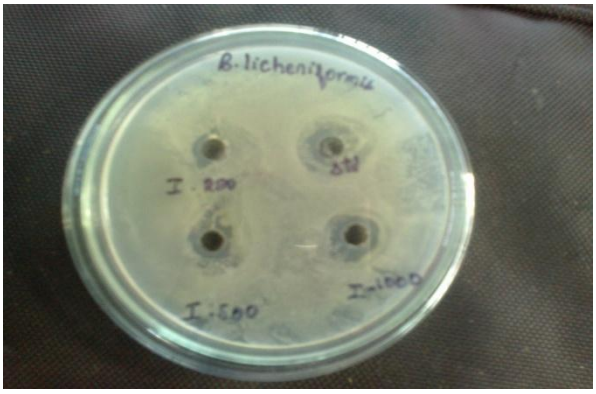


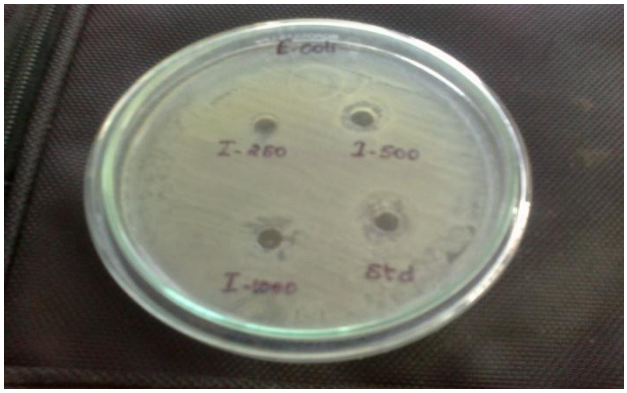
I) 2-(2'-furfural) - 3-hydroxy- 6,8-diiodo -4H- Chromen-4-one

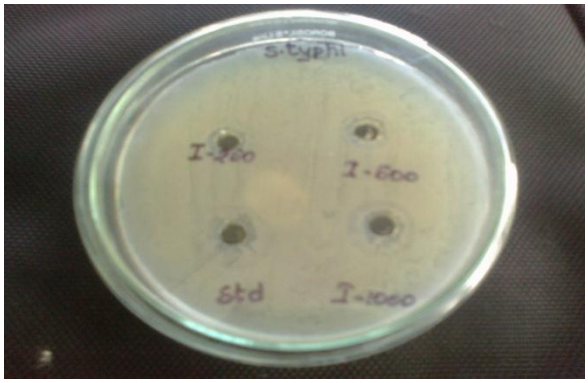


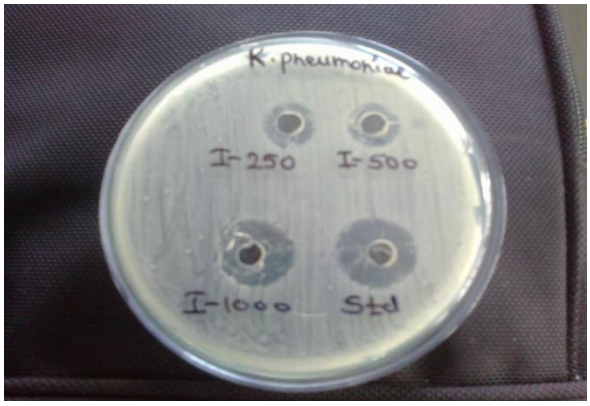












7.2 ANTI-INFLAMMATORY ACTIVITY

7.2.1 *In vitro* Albumin Denaturation Method[65,66]

The synthesized compounds were screened for anti-inflammatory activity using inhibition of albumin denaturation technique. The standard drug and test compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2M,pH 7.4).

Final concentration of DMF in a all solution was less than 2.5%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at $27^o + 1^o$ C for 15 minutes. Denaturation was induced by keeping the reaction mixture at $60^o + 1^o$ C in water bath for 10 minutes.

After cooling, the turbidity was measured at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is taken. The Ibuprofen was used as standard drug.

The percentage inhibition of denaturation was calculated by using following formula.

$$\% \text{ of Inhibition} = 100 \text{ X } (V_t / V_c - 1)$$

Where, V_t = Mean absorbance of test sample.

V_c = Mean absorbance of control.

Table No. 6 Screening of *in-vitro* anti-inflammatory activity.

Compounds	Concentration (µg/ml)	Absorbance	Inhibition of denaturation (%)
Control	-----	0.096	-----
A	100	0.131	36.45
	500	0.171	78.12
B	100	0.134	39.58
	500	0.169	76.04
C	100	0.120	25.00
	500	0.159	65.62
D	100	0.118	22.91
	500	0.157	63.54
E	100	0.124	29.16
	500	0.166	72.91
F	100	0.114	18.75
	500	0.167	73.95
G	100	0.126	31.25
	500	0.165	71.87
H	100	0.132	37.50
	500	0.169	76.04
I	100	0.118	22.91
	500	0.161	67.70
Standard (Ibuprofen)	200	0.188	95.83

All newly synthesized compounds shows good *invitro* anti-inflammatory activity.

In vivo Carrageenan-Induced Paw Edema in rats[69]

Male Wistar albino rats (120-150g) were used in the study, the male rats were divided into four groups (n = 6). The first group received 0.5% CMC (10ml/ kg p.o.), while the second group received indomethacin at the dose level of 10 mg/kg p.o. body weight. The different synthesized compounds at the dose of 50 mg/kg body weight were administered orally to the treated group. Paw edema was induced by the injection of 0.1 ml of 1% freshly prepared suspension of carrageenan into the sub-planter region of the left hind paw of the each group of rat after 30 min. The paw thickness was measured at 0 min, 60 min, 120 min, and 180 min after carrageenan injection by using verniar calipers. The difference between 0 h and subsequent readings were considered as edema volume. The percentage inhibition of edema in various groups was calculated using the formula.

The % inhibition of edema was calculated by following formula.

$$\% \text{ Inhibition of Paw edema} = \frac{(Vt\text{-}Vo)\ \text{control} - (Vt\text{-}Vo)\ \text{treated}}{(Vt\text{-}Vo)\ \text{control}} \times 100$$

Where,

Vt = Paw thickness after carrageenan injection.

V_0 = Paw thickness before carrageenan injection.

The one way ANOVA test followed by Dunnets t test performed by using Graph pad Insat software. The results obtained are showed in the Table.

Table No. 7 Anti-inflammatory activity of compounds in carrageen induced inflammation in rat model.

Treatment	% Inflammation at the time of intervals		Percentage of Inhibition
	0 (h)	3 (h)	
Control (Vehicle) 0.5 % CMC (10 mg/ kg p.o)	2.76 ± 0.006	3.86 ± 0.0011	–
Standard (Indomethacin) (10 mg/kg)	2.58 ± 0.023	2.81 ± 0.05*	79.09
Compound A	2.65 ± 0.01	2.95 ± 0.02*	70.90
Compound B	2.56 ± 0.08	2.90 ± 0.02*	69.09
Compound C	2.54 ± 0.14	2.96 ± 0.12*	61.89
Compound D	2.36 ± 0.01	2.98 ± 0.07*	57.27
Compound E	2.25 ± 0.14	2.85 ± 0.09*	63.63
Compound F	2.58 ± 0.18	2.95 ± 0.12*	66.36
Compound G	2.40 ± 0.08	2.81 ± 0.1*	62.72
Compound H	2.51 ± 0.21	2.83 ± 0.12*	70.90
Compound I	2.48 ± 0.18	2.94 ± 0.09*	59.09

Value expressed as mean ± SEM, *P<0.05 compared with control.

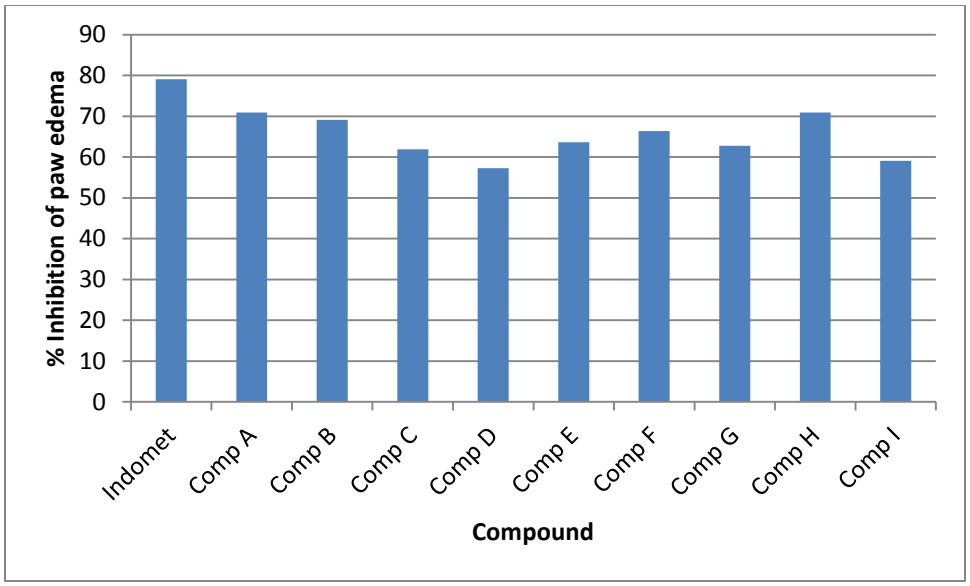


Figure No. 1 Anti-inflammatory activity of Synthesized compounds

7.3 ANTI-DIABETIC ACTIVITY

Selection of animals

Wistar Albino Rats (Male) weighing around 150-200 gm was selected for the experiment. The animals were checked for the free of any disease, only healthy rodent is accepted for the experiments. The male rodents are preferred so that there occurs no interference in between the experiment because of the pregnancy. The rodents are collected from the animal house of Nandha College of pharmacy and Research Institute, Erode 52.

Maintenance of animals

The selected rodents are brought to the laboratory two days before the commencement of the experiment and provided with standard laboratory rodent chow diet obtained from and free access of water, 12hrs day/ dark cycle and room temperature is maintained 27^{o}C. The night before the commencement of the experiment food is withdrawn but free access of water is provided.

Induction of Diabetes

The acclimitatized animals were kept fasting for 24 hrs with water and the initial blood glucose levels were checked and injected with alloxan 150mg/kg dose by intra peritoneal route in normal saline. The blood glucose levels were checked after 72 hrs of alloxan injection. The animals were considered diabetic when the blood glucose level was raised beyond the 200 mg/dL; this condition was observed at the end of 72 hrs after alloxan injection.

Experimental procedure[68]

After confirmation of increased hyperglycemia the diabetic rats were divided into different groups as mentioned below.

Groupings of animals

Group I = Control (Normal saline- 1ml/kg)

Group II = Diabetic Control (Alloxan 150mg/kg)

Group III = Compound F (200 mg/kg)

Group IV = Compound D (200 mg/kg)

Group V = Compound B (200 mg/kg)

Group VI = Compound A (200 mg/kg)

Group VII = Glibenclamide (5 mg/kg)

The drugs were dissolved in normal saline and it was administered orally. Anti-hyperglycemic activity in diabetic rats was assessed by fall in Fasting Blood Glucose level. Blood samples were collected from the tip of the tail on 0^{th}, 1st, 2nd, 3rd week. By without sacrificing the animals, from the tail vein by snipping off the tip of the tail and blood glucose were checked. The biochemical parameters, Cholesterol, Triglycerides, Total protein are determined by using the commercial kit (Ecoline, Manufactured by Merck Specialties, Private limited, Ambernath).

Table No. 8 The effect of Synthesized compounds on fasting blood glucose level in Alloxan induced Diabetic rats

SR.NO	Groups	1st week	2nd week	3rd week
1	Normal control	98.83 ± 6.416**	97.16 ± 5.056**	101 ± 7.572**
2	Diabetes control	255 ± 6.851	270 ± 6.552	297.5 ± 5.737
3	Derivative- I	189.5 ± 7.329**	163.3 ± 5.602**	142.6 ± 6.546**
4	Derivative-II	214.6 ± 5.875**	163.3 ± 5.602**	142.6 ± 6.546**
5	Derivative-III	214.6 ± 9.305**	183.83 ± 9.655**	155.6 ± 3.703**
6	Derivative-IV	216.83 ± 8.920**	188.83 ± 3.790**	160.3 ± 5.765**
7	Standard	183.83 ± 5.307**	149.3 ± 5.283**	139.16 ± 4.453**

Data represents mean ± SEM. (n=6); *p<0.05; **p<0.01

Table No. 9 Effect of Synthesized compounds on RBC, WBC and Hemoglobin of control and experimental rats

SR.NO	Groups	RBC (mm^3 X 10^6)	WBC (mm^3)	Hemoglobin (g dL^{-1})
1.	Normal control	5.725 ± 0.015*	5.106.16 ± 10.26**	9.016 ± 0.06**
2.	Derivative- I	6.05 ± 0.076*	6873.33 ± 12.29*	10.016 ± 0.06*
3.	Derivative-II	6.051 ± 0.04*	6156.66 ± 14.29**	9.8 ± 0.057*
4.	Derivative-III	6.041 ± 0.035*	5806.66 ± 15.42**	9.6 ± 0.051**
5.	Derivative-IV	5.905 ± 0.007*	5493.33 ± 15.42**	9.1 ± 0.057**

Data represents mean ± SEM. (n=6); *p<0.05; **p<0.01

Table No. 10 Effect of Synthesized compounds on Cholesterol, Triglyceride and Total protein of control and experimental rats

SR.NO	Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total protein (g/dl)
1.	Normal control	108.78 ± 4.78	78.35 ± 3.01	7.98 ± 1.41
2.	Derivative- I	114.57 ± 5.03*	81.34 ± 3.67*	7.21 ± 1.21*
3.	Derivative-II	118.28 ± 4.56*	83.69 ± 4.01*	6.94 ± 1.01*
4.	Derivative-III	122.93 ± 4.02*	89.04 ± 3.68*	6.35 ± 0.78*
5.	Derivative-IV	124.27 ± 3.89*	91.82 ± 3.98*	5.88 ± 0.67**

Data represents mean ± SEM. (n=6); *p<0.05; **p<0.01

Table shows the blood glucose levels in rats of different groups. The glucose level was significantly high in Alloxan treated group when compared to that of control and drug treated group. On repeated administration of the test drugs and standard drug for 21 days, a significant decrease in glucose level was observed in diabetic rats. The others biochemical parameters like RBC, WBC and Hemoglobin were found to be increased. The Cholesterol and Triglyceride were also found to be increased while the Total protein were decreased.

8. RESULT AND DISCUSSION

The synthesis of various substituted 3-hydroxy flavones (Compound I to IX) was about 53-83 % yield and was characterized, by the following steps:-

a) Step I - Various acetophenone derivatives like 2-hydroxy acetophenone, *p*-methoxy-hydroxy acetophenone were treated with various aldehydes such as *p*-nitrobenzaldehyde, *p*-chlorobenzaldehyde, furfuraldehyde, anisaldehyde, salicylaldehyde to give respective chalcone derivatives.
b) Step II - Different chalcone derivatives thus obtained were further condensed in presence of hydrogen peroxide and ethanol to yield the desired compounds.
c) Step III - The final flavonol derivative was treated with iodine in presence of methanol to yield the desired compounds.

The Synthesized Compounds were screened for anti-diabetic axtivity. The Compounds F, D, C shows significant activity as compared with that of standard drug. The Compounds having p-methoxy phenyl, 2-hydroxy phenyl and furfural at 2^{nd} position shows better activity when compared with standard Glibenclamide.

Screening of Synthesized Compounds for anti-bacterial activity shows good result from the data mentioned in table no.5. The Compound B, C, F, I afforded better anti-bacterial activity against Gram positive Bacillus substilis, Staphylococcus aureus while Compounds B, F were found potent against Gram negative Escherichia coli, Flavobacterium devorans and Klebsiella pneumonia when compared with standard Streptomycin.

The Synthesized compounds evaluated anti-inflammatory activity by *in vitro* and *in vivo.* The data summarized in table no.6 gives result of *in vitro* anti-inflammatory activity the A, B, E, F, H shows good activity when compared with standard Ibuprofen. The data summarized in table no.7 revaluate the results of *in vivo* anti-inflammatory activity where the Compound A, B, F, H shows good activity when compared to standard Indomethacin.

All the newly synthesized compounds were then identified for their structure and functional groups by using various analytical studies like IR, Mass, ^{1}HNMR spectra's. All the newly synthesized compounds were screened to *in vitro* antimicrobial to various gram positive and gram negative bacteria's using well plate agar diffusion technique by measuring inhibition

zone in millimeters. The anti-inflammatory activity was performed *in- vivo* and *in-vitro*. *In-vitro*, the albumin denaturation technique was used. The Ibuprofen was used as a standard. While, in vivo was performed by carrageenen induced paw edema method. The Indomethacin was used as standard. The both results were correlated by percentage of inhibition. All the synthesized compounds show good anti-inflammatory activity. It is also pharmacological evaluated the synthesized compounds found to be exhibit potent anti-hyperglycemic activity when compared with that of the anti-hyperglycemic standard Glibenclamide at a concentration of 5 mg/kg.

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflamemation[70]. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability or the mediators that increase blood flow[69]. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation whereas prostaglandins are detectable in the late phase of inflammation[71].

It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3 – 4 h after carrageenan injection). Kinin and prostaglandins are involved[75]. Our results revealed that administration of Synthetic Compounds A, B, E, F, H inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

Protein denaturation has been employee as an in vitro screening method for anti-phlogistic agents by Mizushima and his co-workers also confirmed their work and reported that anti-inflammatory drug, inhibit protein denaturation[76,77,78]. Drug binding to plasma albumin may

inhibit thermal denaturation of albumin which perhaps block *Ǿ -NH 2* groups in case of histidine decarboxylase or may displace urate from albumin. All compounds (0.20 mM) showed ability to denature bovine serum albumin, as observed in *in- vitro* inhibition studies. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti inflammation activity, ability of synthesized compound. Protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. As protein denaturation is implicated in inflammation, some compounds which showed good inhibition of denaturation were tested in vivo for anti-inflammatory activity by carrageenan induced edema in the rat paw[71].

The synthesized may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage[73]. Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors[74]. Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the antioxidant and antinflammatory activities of many plants.

Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. Alloxan causes diabetes through its ability to destroy the insulin producing beta cells of the pancreas I studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis.

There is increasing evidence that alloxan caused diabetes by rapid depletion of a cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic states[72]. In this study significant hyperglycemia was achieved within 48 hours after alloxan (150mg/kg b.w. i.p) injection.

Alloxan induced diabetic rats with more than 200mgdl of blood glucose were considered to be diabetic and used for the study.

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Some of the newly synthesized substituted flavonol derivatives were active anti hyperglycemic agent when studied at 200 mg/kg concentration. The evaluated 3-hydroxy flavones derivatives have activity during 21 days whereas F, D, C showed better activity then other compounds, administration of drug in alloxan induced hyperglycemic rats when compared to the standard and control used in the study.

9. SUMMARY AND CONCLUSION

The present study was carried out to synthesize and to screen the newly developed compounds for its pharmacological activity. First attempt of the study was to synthesize the substituted 3-hydroxy flavone derivatives by the Algar-Flynn-Oyamada reaction between acetophenone derivatives like 2-hydroxy acetophenone, 2-hydroxy p-methoxy acetophenone and various aldehydes such as p-nitro benzaldehyde, p-chlorobenzaldehyde, furfuraldehyde, salicylaldehyde, anisaldehyde, to give chalcone products which further condenses in presence of hydrogen peroxide and ethanol to yield the desired flavanol derivatives. Both analytical and spectral data (IR, MS, ^{1}HNMR) of all the synthesized compounds were in full agreement with the synthesized structure.

Flavonoids and phenolic acids have protective role in carcinogenesis, inflammation, atherosclerosis, thrombosis and have high antioxidant capacity. Furthermore, flavonoids have been reported as aldose reductase inhibitors blocking the sorbitol pathway that is linked to many problems associated with diabetes.

Research on flavonoids received an added impulse with the discovery of the French paradox, ie, the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption and a high saturated fat intake. The flavonoids in red wine are responsible, at least in part, for this effect. Furthermore, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease. The association between flavonoid intake and the long-term effects on mortality was studied subsequently and it was suggested that flavonoid intake is inversely correlated with mortality due to coronary heart disease. An important effect of flavonoids is the scavenging of oxygen-derived free radicals. In vitro experimental systems also showed that flavonoids possess anti-inflammatory, anti-allergic, antiviral, and anti-carcinogenic properties.

During the synthetic work the classical preparative organic chemical methods have been applied. The monitoring of the reaction was checked by TLC. Structure elucidation of newly obtained compounds was proved on the basis of the NMR, MS, IR spectra. Chromen-4-ones have been reported to have various pharmacological activities. However, in this study we have

evaluated the compounds (Chromen-4-one) for its *in-vitro* anti-inflammatory, anti-hyperglycemic and anti-microbial activity. The *in-vivo* studies conducted on the alloxan induced diabetes rats indicate that the substituted 3-hydroxy flavones have exhibited marked anti-hyperglycemic activity. The evaluated compounds have better anti hyperglycemic activity for 21 days of drug administration and the compounds like F, D, C showed better activity while other compounds exhibited mild anti hyperglycemic activity. Whereas compounds B, C, F, I afforded better antimicrobial activity against gram positive *Bacillus substilis*, *Staphylococcuss aureus* compound B, F were found to exhibit potent activity against gram negative *Escherichia coli*, *Flavobacterium devorans* and *Klebsiella pneumonia*. The all newly synthesized compounds shows good *in vitro* and *in vivo* anti-inflammatory activity. After comparing the results from pharmacological screening of compounds A to I, it was concluded that the incorporation of a substituited phenyl and furyl moiety in 3-hydroxy flavone or flavanol ring enhances their pharmacological activity. The activity of the compounds depends upon the nature and the position of the substitution at 3 and 7 of chromen-4-one moiety. Also, the substitution with p-nitrophenyl, p-chlorophenyl, furyl, 2-hydroxyphenyl and p-methoxyphenyl at the 2^{th} position and 7^{th} position with methoxy, showed pronounced *in-vitro* anti-inflammatory, anti hyperglycemic and antimicrobial activity.

The mechanisms and the sequence of events by which free radicals interfere with cellular functions are not fully understood, but one of the most important events seems to be lipid peroxidation, which results in cellular membrane damage. This cellular damage causes a shift in the net charge of the cell, changing the osmotic pressure, leading to swelling and eventually cell death. Free radicals can attract various inflammatory mediators, contributing to a general inflammatory response and tissue damage. To protect themselves from reactive oxygen species, living organisms have developed several effective mechanisms. The antioxidant-defense mechanisms of the body include enzymes such as superoxide dismutase, catalase, and glutatione peroxidase, but also nonenzymatic counterparts such as glutathione, ascorbic acid, and α-tocopherol. The increased production of reactive oxygen species during injury results in consumption and depletion of the endogenous scavenging compounds. Flavonoids may have an additive effect to the endogenous scavenging compounds. Flavonoids can interfere with ≥3 different free radical–producing systems, which are described below, but they can also increase the function of the endogenous antioxidants. The present study on the evaluation of 4-flavones

derivatives (flavanol) were found in significant activity of *in vitro* and *in vivo* anti-inflammatory, anti-microbial and anti-hyperglycemic. The study of flavonoids is complex because of the heterogeneity of the different molecular structures and the scarcity of data on bioavailability. Furthermore, insufficient methods are available to measure oxidative damage in vivo and the measurement of objective endpoints remains difficult. There is a need to improve analytic techniques to allow collection of more data on absorption and excretion. Data on the long-term consequences of chronic flavonoid ingestion are especially scarce. In conclusion, the in vivo studies that have been performed do give a hopeful picture for the future

On the basis of order of pharmacological studies of test compounds the following structure activity relationship (SAR) was observed

1. At C-2, p-nitrophenyl, p-chlorophenyl, p-methoxyphenyl, p-hydroxyphenyl substitution showed the best activity followed by furyl substitution.

2. Non substitution of flavonol ring at C-7 was less favorable for the activity than C-7 methoxy and 6,8 di-iodo substitution.

3. At C_{-4}' substitution of C-2 phenyl ring was more favorable.

10. REFERENCES

1) J.H. Block, Wilson and Grisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, Lippincott Williams and Wilkins, Baltimore. 11th Edition, 2004, 1-2.
2) W.O. Foye, Principles of Medicinal Chemistry, Wolter Klwer Health India(Pvt) Ltd, New Delhi, Baltimore, 6th Edition, 2008, 1-2,1147-1154.
3) P.Venkatesan and T.Maruthavanan, Asian Journal Of Chemistry, 23, 5(2011), 2121-2124.
4) W.O. Foye, Principles of Medicinal Chemistry, Wolter Klwer Health India(Pvt) Ltd, New Delhi, Baltimore, 3th Edition, 1989, 1.
5) S.S Lim, H.S Kim and D.U Lee, Bulletin Korean Chemical Society, 28, 12, 2007, 2495-2497.
6) Remington's, The Science and Practice of Pharmacy, Lippincott Williams and Wilkins, 21st Edition, 2005, 439, 1718.
7) T.P Cushnie, International Journal Of Antimicrobial Agents, 26, 2005, 343-356.
8) K.R Narayana, M.S Reddy, M.R Chaluvadi, and D.R Krishna, Indian Journal of Pharmacology, 2001, 33, 2-16.
9) B. Havsteen, Biochemical Pharmacology, 1983, 32(7), 1141-1148.
10) E. Middleton, Trends in Pharmacological Sciences, 1984, 5, 335-338.
11) J.A Joule, Heterocyclic Chemistry, Keith Milles, 4th Edition, 170-191.
12) D. Amic, D.D Amic, D. Beslo, N. Trinajstic, Croatica Chemica Acta, 76(1), 2003, 55-61.
13) Y.C Xu, W.S Leung, D.K.Y Yeung, L.H Hu, G.H Chen, C.M Che, R.Y.K Man, Phytochemistry, 68, 2007, 1179-1188.
14) J.W Chen, Z Qin, T Hu,, D Zhua, Chinese Academy of Sciences, China, 23(7), 2003, 667-672.
15) H.S Rosenkranz, B.P Thampatty, Department of Biomedical Sciences, USA, 13(2), 2003, 529-535.
16) Z. Li, G. Ngojeh, P.D Witt, Z Zhang, M. Chen, B. Lainhart, V. Li, P. Felpo, Tetrahedron, 49, 2008, 7243-7245.
17) C.B Patil, S.K Mahajan, S.A Katti, Journal of Pharma Science and Research,1(3), 2009, 11-22.

18) H. Zhang, Y. Sun, D. Chen, Chinese Chemical Letters, 13(6), 2002, 531-534.

19) H.P Rang and M.M Dale, Pharmacology, Churchill Livingstone, UK, 6th Edition, 2007, 397-408.

20) M.N Dravid, The Pathophysiology of Diabetic Complication, 174, 1996, 286-289.

21) N. Maclean, R.F Ogalive, Diabetic Ketoacidosis, Current Views on Pathogenesis and Treatment Diabetologia, 29, 1986, 71-77.

22) H Bondansky, The Natural History of Diabetes, Practical Diabetes, 6(1), 1989, 29.

23) D. Manzella, Health's Disease and content is reviewed by our Medical Review Board 01.About.com, 2009, 105.

24) R.L Magnuson, D.K Granner, Mammalian Glucokinase, Annual Review of Nutrition, 13, 1993, 463-496.

25) R.A Harvey, Antiinflamatory Drugs, Pharmacology, Lippincott Williamss and Wilkins, 2nd Edition, 401-403.

26) S.K Gupta, Drug Screening Methods, Jaypee Brother's Medical Publisher's(Pvt) Ltd, New Delhi, 1st Edition, 2004, 481-497.

27) R. Dale, Local Harmones, Inflammation and Immune Reactions, Pharmacology, 6th Edition, 2007, 202-203,213.

28) M. Dharmishtha, G. Falguni, International Journal of Pharmatech Research, 1, 2009, 679-681.

29) C. Junghanss, K.A Marr, R.A Carter , B.M Sandmaier, M.B Maris, D.G Maloney, T. Chauncey, P.A Mesweeney, R Storb, Biology of Blood and Bone Marrow Transplantation, 8, 2002, 512-520.

30) T.P Cushnie, International Journal Of Antimicrobial Agents, 26, 2005, 343-356.

31) B. Ozcelik, D.D Orhan, S. Ozgen, F. Ergun, Tropical Journal of Pharmaceutical Research, 7, 2008, 1151-1157.

32) K.R Narayana, M.S Reddy, D.R Krishna,Indian Journal Of Pharmacology, 2001, 33, 2-16.

33) W.R Bansal, R. Kumar, Indian Journal Of Chemistry, 46A, 2007, 1440-1444.

34) Z.Li, G. Ngojeh, M. Chen, B.Lainhart, V.Li, P.Felpo, Tetrahedron, 49, 2008, 7243—7245.

35) S.B Olaleye, J.Moke, A.K Etu, Nigerian Journal Of Physiological Sciences, 19,1, 2004, 69-76.

36) M.G de Carvalho,V.C de Silva, T.M de Silva, Department of Quimica, Brasil, 44,34-37.

37) A.R Tapas, D. M Sakarkar, R.B Kakde, Tropical Journal Of Pharmaceutical Research, 2008, 7(3), 1089-1099.

38) D. Amic, D. Beslo, N. Trinajstic, Croatica Chemica Acta,76,1, 2003, 55-61.

39) M.A Alvarez, N.B Debattista, N.B Pappano, Folia Microbiology, 53, 1, 2008, 23-28.

40) J.J Xue, X.S Zhang, X.Z Liang, Y. Li, Chinese Chemical Letters, 14, 5, 2003, 443-444.

41) C. Carlos, L. Alarcon Julio, M. Hafidi, Boletin Latinoamericano Y Del Caribe De Plantas Medicinales Y Aromaticas Santiago, Chile, 9, 6, 2010, 432-439.

42) N. Bhaskar, M.K Reddy, Journal Of Chemical And Pharmaceutical Research, 2011, 3(3), 759-765.

43) W. Bylka, I. Matlawska, N.A Pilewski, Journal Of American Nutrition Association, 7, 2, 2004, 24-31.

44) A. Boumendjel, Journal Of Medicinal Chemistry, 51, 2008, 2307-2310.

45) A. Nambi, S. Viswanathan, Indian Journal Of Pharmaceutical Science, 58, 1, 1996, 18-21.

46) C. Conti, P. Tomao, D. Genovese, N. Desideri, M.L Stein and N. Orsi, Antimicrobial agents and Chemotherapy, 36, 1, 1992, 95-99.

47) B. Jayashree, A. Alam, Indian Journal Of Heterocyclic Chemistry, 19, 2010, 237-240.

48) T. Sivakumar, M. Gupta, Journal Of Pharmalogical Science, 94, 2004, 177-184.

49) A.H Chen, W.B Kuo and C.W Chen, Journal Of Chinese Chemical Society, 2003, 50, 123-127.

50) K.N Mewett, S.P Fernandez and A.K Pasricha, Bioorganic And Medicinal Chemiatry Journal, 17, 2009, 7156-7173.

51) D.S Ghotekar, P. Mandhane, Indian Journal Of Heterocyclic Chemistry, 19, 2009, 101-104.

52) V.M Gorduza, C.T Mihaila, C. Cernatescu and M. Rusa, Ovidus University Annals Of Chemistry, 11, 2000, 56-59.

53) Y.B Vibhute, S.S Mokle, S.V Khansole and R.B Patil, International Journal Of Pharma And Bio Sciences, Nanded(M.S), India, 1, 2010,1-7.

54) B. Sharma, Asian Journal Of Chemistry, New Delhi, 21, 6, 2009, 4233-4236.

55) B.B Wankhade, V. N Saoji, D.R Chaplet and C.M Bharambe, Malkapur, India, Asian Journal Of Chemistry, 23, 4, 2010, 2801-2804.

56) A. Kamal, R. Ramu, G.B Khanna, A.K Saxena, M. Shanmugavel and R.M Pandita, Journal of Biochemistry and Molecular Biology 38, 2005, 83-91.

57) Z.J Witezak, Pure and Applied Chemistry, IUPAC, 66, 10/11, 1994, 2189-2192.

58) S.R Pattan, S.G Jadhav, Indian Journal Of Heterocyclic Chemistry, 17, 2007, 23-26.

59) D.H Zhao, X. Sui and L.P Guan, China, Asian Journal Of Chemistry, 23,3, 2011, 1129-1132.

60) P. Venkatesan and T. Maruthavanan, Salem(T.N), India, Asian Journal Of Chemistry, 23, 5, 2011, 2121-2124.

61) S. Tachakittirungrod, F. Ikegami and S. Okonogi, Japan, Scientia Pharmaceutical, 75, 2003, 179-193.

62) K.K Marcel, M. Bekro and Z.B Jerome, Journal Of Pharmagnosy and Phytotherapy, 3, 2011, 8-12.

63) J. Pogany, pogany@t-online,hu, Pretoria, South Africa, 2005, 25.

64) Vogel's, Text book of Practical Organic Chemistry, 5th Edition, 2004, 1203.

65) B.S Sathe, International Journal of Pharmacy and Pharmaceutical Sciences, 3, 2011, 220-222.

66) S.K Sangal and P.K Rastivona, International Journal of Pharma and Bio Sciences, 1986, 104, 3402.

67) G. Kumar, P. Mishra and V. Prakash, Global Journal of Biotechnology and Biochemistry, 4(1), 2009, 37-42.

68) A. Panthong, D. Kanjanapothi, Phytomedicine, 1, 1994, 141-144.

69) A. Ialenti, A. Ianaro, S. Moncada, M.D Rosa, European Journal of Pharmacology, 1995, 211, 177-184.

70) R.N Mitchell, R.S Cotran, In: Robinsons Basic Pathology, ed 7. Harcourt Pvt. Ltd, New Delhi, India, 2000, 33-42.

71) D. Salvemini, Z.Q Wang, D.M Bourdon, M.K Stern, M.G Currie, P.T Manning, European Journal of Pharmacology, 303, 1996, 217-224.

72) P.M Hernandez, R Rabanal Gallego, Journal of Ethnopharmacology, 81, 2002, 43-47.

73) K Yasodha, K.N Jayaveera, K.R Reddy, K Rupesh, D Raghavendra, M Siddaiah, Journal of Pharmacy and Chemistry, 2008, 156-160.

74) C.T Chou, Phytochemical Research, 11, 1997, 152-154.

75) S.N Das, S Chatterjee, Indian Indigenous Medicine, 16(2), 1995, 117-123.

76) N.H Grant, H.E Alburn and A Kryzanauskas, Biochemical Pharmacology, 19, 1970, 715-722.

77) Y Mizushima,Archieves of International Pharmacodynamic, 149, 1964, 1-7.

78) Y Mizushina and M Kobayashi, Journal Pharmacy and Pharmacology, 20, 1968, 169-171.